















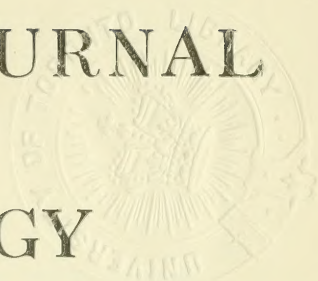


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## ACIDOSIS DURING STARVATION

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### INTRODUCTION

The physiological effects of starvation have been investigated by numerous observers and many papers have been published on the subject. Castellino noted a decrease in the alkalescence of the blood in starving rabbits in 1893, and in the following year Tanszk observed the same phenomenon in Succì's blood during a period of starvation. This result was confirmed by the careful studies of Benedict (1) in 1907. London also observed a slight decrease in blood alkalescence in starving rabbits. The appearance of acetone bodies in urine and expired air, which is characteristic of the late stages of starvation, has also been extensively studied. The output of carbon dioxide is said by various authors to show a steady reduction throughout periods of inanition. Thus Benedict reports that the carbon dioxide content of the alveolar air was subnormal on the second day of a fast. Thereafter it held constant until the fourteenth day, when a second drop occurred, after which it suffered no further reduction. In each case the fall in tension amounted to about 4 mm. Hg. Thus it may be inferred that a tendency toward acidosis developed in this individual on the second day and that this tendency toward acidosis was accentuated on the fourteenth day.

We know that the hydrogen ion concentration of the blood rather than the carbon dioxide tension is the predominating factor in the control of respiration, and also that the acidity of the blood may be divided into two parts, one due to carbon dioxide and another one

due to other acids. As the total hydrogen ion concentration necessary to stimulate the respiratory center must always be the same, it is readily seen that if the other acids in the blood increase in amount, the tension of carbon dioxide will decrease. Since alveolar carbon dioxide tension represents closely the carbon dioxide tension in the arterial blood, it affords a good index of the acidity of the blood. Therefore this index may be much more satisfactory and important than the urinary tests for acidity.

Van Slyke and Cullen (2) have recently adopted the plasma bicarbonate as the ideal measure for the alkaline reserve of the blood; a criterion much better than the measurement of the alveolar carbon dioxide tension for the purpose of determining "acidosis" in the modern broader sense. For this determination they published a new method and designed a suitable apparatus.

TABLE 1  
*Influence of two extractions*

| DAYS OF FAST.....<br>ANIMAL NUMBER.....  | 3<br>7           | 4<br>9           | 7<br>16          | 9<br>12          | 11<br>25         |
|--|------------------|------------------|------------------|------------------|------------------|
| CO <sub>2</sub> in 1 cc. arterial plasma {cc. ....<br>{mgm..                   | 0.3642<br>0.7150 | 0.3522<br>0.6911 | 0.3369<br>0.6616 | 0.1723<br>0.3377 | 0.2204<br>0.4328 |
| CO <sub>2</sub> of the same animal pre-<br>ceding day..... {cc. ....<br>{mgm.. | 0.5170<br>1.015  | 0.4028<br>0.7902 | 0.4112<br>0.8071 | 0.3960<br>0.7778 | 0.3268<br>0.6419 |

TABLE 2  
*Influence of second extraction on moribund animals*

| DAYS OF FAST.....<br>ANIMAL NUMBER.....                         | 5<br>11          | 14<br>23         | 20<br>17         |
|---|------------------|------------------|------------------|
| CO <sub>2</sub> in 1 cc. of arterial plasma {cc. ....<br>{mgm.. | 0.4486<br>0.8812 | 0.4280<br>0.8406 | 0.4468<br>0.8770 |

TABLE 3  
*Arterial plasma bicarbonate in short time after death*

| DAYS OF FAST.....<br>ANIMAL NUMBER..... | 12<br>24        | 14<br>26         | 15<br>23         | 18<br>27         |
|---|-----------------|------------------|------------------|------------------|
| CO <sub>2</sub> {cc. ....<br>{mgm.....  | 0.5907<br>1.101 | 0.5837<br>1.1467 | 0.7220<br>1.4182 | 0.8583<br>1.6860 |



TABLE 4  
*Arterial plasma bicarbonate during starvation*

| DAYS         | ANIMAL<br>NUMBER | CO <sub>2</sub> | CO <sub>2</sub> | ANIMAL<br>NUMBER | CO <sub>2</sub> | CO <sub>2</sub> |
|--------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|
|              |                  | cc.             | mgm.            |                  | cc.             | mgm.            |
| Normal       | 10               | 0.5495          | 1.078           | 18               | 0.5666          | 0.113           |
| Days of fast |                  |                 |                 |                  |                 |                 |
| 1            | 20               | 0.4938          | 0.9690          |                  |                 |                 |
| 2            | 7                | 0.5170          | 1.015           |                  |                 |                 |
| 3            | 9                | 0.4028          | 0.7902          |                  |                 |                 |
| 4            | 11               | 0.4096          | 0.8040          |                  |                 |                 |
| 5            | 15               | 0.4088          | 0.8026          |                  |                 |                 |
| 6            | 16               | 0.4112          | 0.8071          |                  |                 |                 |
| 7            | 12               | 0.3960          | 0.7778          |                  |                 |                 |
| 8            |                  |                 |                 |                  |                 |                 |
| 9            | 17               | 0.3830          | 0.7518          |                  |                 |                 |
| 10           | 25               | 0.3268          | 0.6419          |                  |                 |                 |
| 11           | 24               | 0.3423          | 0.6722          |                  |                 |                 |
| 12           |                  |                 |                 |                  |                 |                 |
| 13           | 28               | 0.3469          | 0.6813          |                  |                 |                 |
| 14           |                  |                 |                 |                  |                 |                 |
| 15           |                  |                 |                 |                  |                 |                 |
| 16           | 27               | 0.3723          | 0.7311          | 21               | 0.2720          | 0.5141          |

As was shown by several authors, it is doubtless a fact that in the blood during fasting an acidosis develops. I have wished to determine the acidosis precisely by Van Slyke and Cullen's method and found in the blood plasma of 28 rabbits a similar fall of bicarbonate as reported by Benedict (3) on the finding of Levanzin's alveolar air.

This research was begun in January, 1918, at the Institute for Forensic Medicine of the Tokyo Imperial University and concluded in May, 1918. A preliminary report of the work was made at the Forensic Medical Department of the Fifth Japanese Medical Congress in April, 1918. I desire at this time to express my hearty thanks to Prof. Dr. K. Katayama and Prof. Dr. S. Mita for their kind assistance in the direction of my experiments.

#### MATERIAL AND TECHNIQUE

I collected the arterial blood by means of a cannula tube, allowing it to flow into potassium oxalate solution under a layer of paraffin oil. The oxalate solution was of strength such that the salt amounted to about 0.5 per cent of the blood in the tube. The blood thus drawn

was centrifuged immediately and 1.0 cc. of the plasma was subjected to the determination, according to Van Slyke and Cullen's description. The determination was carried out two or three times on one and the same sample and the average of these was adopted as a result.

#### RESULTS

Of 28 rabbits used in these experiments, the results of nos. 1 to 6 were discarded because of technical errors. Nos. 8, 13, 14, 19, 21 and 22 died in the course of the fast, consequently their blood plasma bicarbonate could not be determined. The blood of nos. 23 and 26 was taken immediately after their death and subjected to determination. The remainder, i.e., 14 rabbits, were examined before death. The plasma bicarbonate is remarkably influenced by the condition of the animals as the following results will show.

TABLE 5  
*Loss of body weight*

| DAYS OF FAST | NUMBER OF ANIMALS |      |      |      |      |      |      |
|--------------|-------------------|------|------|------|------|------|------|
|              | 9                 | 11   | 15   | 16   | 19   | 7    | 8    |
| 1            | 2024              | 2328 |      | 2150 | 2000 |      | 1924 |
| 2            | 1910              | 2190 | 2012 | 2134 | 1848 | 1518 | 1820 |
| 3            | 1860              | 2150 | 1940 | 2126 | 1764 | 1380 | Dead |
| 4            | 1790              | 2075 | 1872 | 1934 |      |      |      |
| 5            |                   | 2000 | 1812 | 1920 | Dead |      |      |
| 6            |                   |      | 1764 | 1904 |      |      |      |
| 7            |                   |      | Dead | 1904 |      |      |      |

Italic figures indicate the first extraction of blood and heavy face figures the second extraction. Animals were not permitted to survive the second bleeding.

#### *1. Influence of various conditions on the arterial plasma bicarbonate*

*a. Two or more extractions of blood from carotid.* As seen in table 1, the plasma bicarbonate is enormously reduced by the second bleeding in animals yet capable of surviving for several days. But in the case of an animal moribund from inanition, the plasma bicarbonate, on the contrary, is more or less increased, as shown in table 2.

*b. In short time after death.* Shortly after death the arterial plasma bicarbonate definitely increased as compared with the moribund state. This applies equally for the first as for the second bleeding, as is evident from table 3.

## *2. The amount of plasma bicarbonate in the arterial blood of fasting rabbits*

As seen in table 4, the arterial plasma bicarbonate is about 55 volume per cent before the fast, about 50 on the first and second days of the fast, and from the third to the ninth day it falls to a level of about 40 volume per cent; afterwards there occurs a third rather moderate fall, namely, to about 34 volume per cent which remains without any further change until the sixteenth day, when death usually occurs.

## *3. Loss of body weight during the fast*

As presented in tables 5 and 6, the body weight of most experimental animals is seen to be remarkably reduced on the second day of the fast, but the rate of the weight loss is subjected to an extreme fluctuation on account of individual variations and is not necessarily influenced by drawing of blood. The 11 rabbits in table 6, i.e., nos. 12, 17 and 20 to 28 were left in the state of absolute fast until death. They lived for 11 to 20, in the average 14.5 days and lost in the end 27.69 to 52.37, in the average 41.78 per cent of their initial body weight, the same as recorded by several authors. There seems to exist no special relationship between the loss of body weight and the fall in plasma bicarbonate. The more or less conspicuous loss of body weight on the second day of the fast which was often but not always observed, seemed to have some relationship with the first fall in arterial carbon dioxide tension; the second and third falls, however, were accompanied by no precipitate loss of weight.

## *4. Finding by autopsy*

Although the experimental animals lost markedly in weight, the stomachs were invariably found to contain a considerable amount of grayish fecal material. Gross changes were seldom apparent in the organs; coccidiosis of liver and intestinal ulcers with bleeding was noted in no. 24, and intra-muscular hemorrhages were found in no. 16. The urine was usually clear and acid to litmus.

Microscopically I found intensive congestion in small arteries and capillaries of every organ (brain, lung, liver, spleen and kidney) and cloudy swelling, vacuolarization and atrophy of various degree in the parenchymatous cells of liver and kidney. None of my preparations show fatty degeneration except in ovary and adrenal. Hemosiderosis of liver cells and pigmentation of spleen were strikingly obvious in animals after 10 days' fast.

*Loss of body weight in more than ten days' fast*

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## CONCLUSIONS

1. On the first and second days of starvation, the plasma bicarbonate in the arterial blood of rabbits showed a drop from the normal value. On the third day of the fast there was a second rather sharp fall, after which there was no change until the ninth day. On the tenth fasting day there occurred a third rather moderate fall, after which no further marked change took place until the end of life. Generally the arterial plasma of rabbits has 55 volume per cent of carbon dioxide. The first fall is about 5 volume per cent, the second about 10 and the third about 6 volume per cent.

2. The amount of carbon dioxide in the arterial plasma is influenced considerably by the condition of the animals. After one extraction of 10 cc. blood from the carotid the acidosis seems to be conspicuously increased, because by the second extraction the amount of carbon dioxide is always less than that of the first extraction on the same fasting day.

In the moribund state a contrary result is obtained, i.e., the amount of carbon dioxide in the arterial plasma does not decrease but increases. This is also the case in the arterial plasma immediately after death. This increase is, I think, not due to bicarbonate, but rather to an accumulation of carbon dioxide caused by the failure of the circulatory as well as the respiratory functions.

3. The rate of the loss of body weight is subjected to wide individual fluctuations which may or not may be influenced by the blood extraction. The animals lived in the state of an absolute fast for 11 to 20, in the average 14.5 days and at the end of life had lost 27.69 to 52.37, in the average 41.78 per cent of their initial weight.

There is no demonstrable relationship between the loss of body weight and the fall in amount of plasma bicarbonate.

4. Microscopically many organs showed cloudy swelling, vacuolization and atrophy. There was invariably an intensive congestion in every glandular organ, but fatty degeneration was found almost in no case.

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# STUDIES ON THE EXTRACT OF LUNG

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## INTRODUCTION

Brieger and Uhlenhuth (1) reported that the hypodermic injection of tissue extract into guinea pigs caused death. More recently Kraus and Volk (2) stated that the intravenous injection of the extract of tuberculous tissue killed guinea pigs. Dold (3), (4) reported further that the extract of normal lung, injected intravenously into rabbits and guinea pigs, caused death and it was also found by him that the toxin involved in this reaction was neutralized by normal fresh serum. Since then various authors (5), (6), (7), (8), (9), (10), (11), (12), (13), (14), (15), (16) have studied the nature and property of this tissue toxin, but their results have been very contradictory. Thus it may be suspected that the tissue toxin in itself is not a simple body. I have investigated the toxin-neutralizing power of a number of substitutes and also studied the influence of the toxin upon the sugar content of the blood.

## METHOD OF PREPARING THE LUNG EXTRACT

*a. The extract of beef lung.* From one to two hours after death, the lung was minced and five parts in weight were mixed to seven volumes of 0.85 per cent sodium chloride solution and allowed to stand two hours in the cold. Then it was passed through lint and the filtrate was centrifuged until the supernatant fluid was free of solid matter. This supernatant fluid was used as the lung extract.

*b. The extract of the lungs of rabbits and guinea pigs.* Immediately after the death caused by bleeding the lung was taken out and was extracted in the same way as above.

## SYMPTOMS OF INTOXICATION IN GUINEA PIGS

All the experiments on the toxic action of the lung extract were carried out on guinea pigs and the injection of the extract was done

into the external jugular vein. When a lethal dose of the extract was thus given intravenously a large majority of the animals were much stimulated, urinated and stooled, had a general clonic convulsion, after which the respiration was labored and in two or three minutes coma developed followed shortly by death. In other cases the symptoms were not so typical; the latent period of the reaction was prolonged and death did not follow for an hour or two hours. When sublethal doses were given, some of the animals were thrown into convulsions, which were followed by labored respiration as above and then after a few minutes to many hours were completely restored; others showed only a transitory dyspnoea. Subcutaneous and intraperitoneal injections produced no acute symptoms.

#### TOXICITY OF LUNG EXTRACT

The minimal lethal dose of beef lung extract was determined upon guinea pigs by intravenous injection. The extract was always freshly made. In the table (table 1) the notation (+) indicates death within one hour; the notation (—), failure of the dose to kill. Because of variability in the resistance of guinea pigs, each dose was tested on two animals and the minimal dose sufficient to kill both animals was taken as the minimal lethal dose. In the table this has been expressed in cubic centimeters per 100 grams body weight.

As table 1 shows, the toxin varied in potency, the minimal lethal dose on guinea pigs with intravenous injection ranging from 0.02 to 0.15 cc. per 100 grams of the body weight.

#### ON THE INFLUENCE OF GLUCOSE UPON THE TOXICITY OF THE LUNG EXTRACT

The observation by Dold that a fresh serum neutralized the tissue toxin has been confirmed by many authors. In this connection I undertook to study the influence of 10, 15 and 42 per cent solutions of grape sugar upon the potency of the toxin. In these experiments glucose and extract of beef lung were mixed in vitro in the proportions shown in the following table (table 2) and injected into the external jugular vein of guinea pigs.

TABLE 1  
*The toxicity of the beef lung extract on guinea pigs*

| DATE<br>(1918) | BODY<br>WEIGHT | SEX | AMOUNT OF<br>EXTRACT IN-<br>JECTED PER<br>100 GRAMS<br>OF BODY<br>WEIGHT | RESULT | TIME FROM<br>INJECTION<br>TO DEATH |
|----------------|----------------|-----|--|--------|------------------------------------|
|                | <i>grams</i>   |     | <i>cc.</i>   |        | <i>minutes</i>                     |
| July 9.....    | 380            | ♂   | 0.06   | +      | 8                                  |
|                | 400            | ♂   | 0.03   | +      | 6                                  |
|                | 380            | ♀   | 0.03   | +      | 50                                 |
|                | 410            | ♂   | 0.02   | +      | 5                                  |
|                | 380            | ♂   | 0.02   | +      | 12                                 |
|                | 390            | ♂   | 0.02   | -      |                                    |
|                | 400            | ♂   | 0.01   | -      |                                    |
| July 11.....   | 450            | ♀   | 0.02   | -      |                                    |
|                | 370            | ♀   | 0.02   | -      |                                    |
|                | 370            | ♂   | 0.02   | -      |                                    |
|                | 350            | ♂   | 0.03   | +      | 50                                 |
|                | 320            | ♀   | 0.03   | +      | 6                                  |
| July 17.....   | 440            | ♀   | 0.03   | -      |                                    |
|                | 600            | ♀   | 0.03   | -      |                                    |
|                | 610            | ♀   | 0.03   | -      |                                    |
|                | 640            | ♀   | 0.04   | -      |                                    |
|                | 570            | ♀   | 0.04   | -      |                                    |
|                | 550            | ♀   | 0.05   | -      |                                    |
|                | 610            | ♀   | 0.05   | -      |                                    |
|                | 830            | ♀   | 0.1  | +      | 18                                 |
|                | 500            | ♀   | 0.1  | +      | 12                                 |
|                | 580            | ♀   | 0.1  | +      | 38                                 |
| July 20.....   | 430            | ♂   | 0.03   | -      |                                    |
|                | 450            | ♀   | 0.03   | -      |                                    |
|                | 470            | ♀   | 0.05   | +      | 25                                 |
|                | 510            | ♀   | 0.05   | +      | 16                                 |
|                | 450            | ♂   | 0.1  | +      | 17                                 |
| August 9.....  | 400            | ♂   | 0.01   | -      |                                    |
|                | 510            | ♀   | 0.01   | -      |                                    |
|                | 610            | ♀   | 0.02   | +      | 11                                 |
|                | 520            | ♂   | 0.02   | +      | 15                                 |
|                | 490            | ♂   | 0.05   | +      | 3                                  |
| August 10..... | 300            | ♀   | 0.05   | -      |                                    |
|                | 400            | ♂   | 0.08   | -      |                                    |
|                | 500            | ♀   | 0.1  | +      | 3                                  |
|                | 570            | ♀   | 0.1  | +      | 45                                 |

TABLE 1—*Concluded*

| DATE<br>(1918)    | BODY<br>WEIGHT | SEX | AMOUNT OF<br>EXTRACT IN-<br>JECTED PER<br>100 GRAMS<br>OF BODY<br>WEIGHT | RESULT | TIME FROM<br>INJECTION<br>TO DEATH |
|-------------------|----------------|-----|--|--------|------------------------------------|
|                   | <i>grams</i>   |     | <i>cc.</i>   |        | <i>minutes</i>                     |
| August 12.....    | 410            | ♂   | 0.02   | —      |                                    |
|                   | 420            | ♂   | 0.05   | —      |                                    |
|                   | 420            | ♂   | 0.05   | +      | 10                                 |
|                   | 420            | ♂   | 0.1  | +      | 15                                 |
|                   | 550            | ♀   | 0.1  | +      | 45                                 |
|                   | 380            | ♂   | 0.15   | +      | 55                                 |
|                   | 440            | ♀   | 0.15   | +      | 5                                  |
|                   | 490            | ♀   | 0.2  | +      | 9                                  |
| September 2.....  | 400            | ♀   | 0.1  | +      | 5                                  |
|                   | 500            | ♀   | 0.05   | +      | 13                                 |
|                   | 290            | ♀   | 0.05   | +      | 50                                 |
|                   | 520            | ♀   | 0.02   | +      | 5                                  |
|                   | 500            | ♀   | 0.02   | +      | 5                                  |
|                   | 500            | ♂   | 0.01   | —      |                                    |
|                   | 570            | ♀   | 0.01   | —      |                                    |
| September 11..... | 400            | ♀   | 0.02   | —      |                                    |
|                   | 500            | ♂   | 0.02   | +      | 9                                  |
|                   | 490            | ♀   | 0.05   | +      | 6                                  |
|                   | 460            | ♂   | 0.05   | +      | 55                                 |
|                   | 450            | ♀   | 0.08   | +      | 5                                  |
|                   | 520            | ♀   | 0.1  | +      | 4                                  |
|                   | 360            | ♀   | 0.1  | +      | 20                                 |
| September 14..... | 510            | ♀   | 0.05   | —      |                                    |
|                   | 440            | ♂   | 0.1  | —      |                                    |
|                   | 360            | ♀   | 0.1  | —      |                                    |
|                   | 360            | ♂   | 0.15   | +      | 3                                  |
|                   | 420            | ♀   | 0.15   | +      | 7                                  |
| September 17..... | 400            | ♂   | 0.05   | —      |                                    |
|                   | 380            | ♂   | 0.1  | +      | 55                                 |
|                   | 420            | ♂   | 0.1  | +      | 40                                 |
|                   | 450            | ♂   | 0.15   | +      | 34                                 |
|                   | 510            | ♀   | 0.15   | +      | 19                                 |
|                   | 390            | ♀   | 0.2  | +      | 4                                  |



TABLE 2

*The influence of glucose solution upon the toxicity of the beef lung extract on guinea pigs*

| DATE<br>(1918) | BODY<br>WEIGHT | SEX | AMOUNT OF GLUCOSE<br>MIXED | AMOUNT<br>OF NaCl<br>SOLUTION<br>(0.85 PER<br>CENT)<br>MIXED | AMOUNT<br>OF LUNG<br>EXTRACT<br>PER 100<br>GRAMS OF<br>BODY<br>WEIGHT | RESULT | TIME FROM<br>INJECTION<br>TO DEATH |    |
|----------------|----------------|-----|----------------------------|--|---|--------|------------------------------------|----|
|                | grams          |     | cc.                        | cc.  | cc.   |        | minutes                            |    |
| July 11.....   | 350            | ♂   | 10 per cent solu-<br>tion  |  | 0.03  | +      | 50                                 |    |
|                | 320            | ♀   |                            |  | 0.03  | +      | 6                                  |    |
| July 11.....   | 400            | ♂   |                            |  | 0.03  | +      | 8                                  |    |
|                | 400            | ♀   |                            |  | 0.03  | —      |                                    |    |
|                | 380            | ♀   |                            |  | 0.03  | —      |                                    |    |
|                | 310            | ♂   |                            |  | 0.45  | —      |                                    |    |
|                | 360            | ♀   |                            |  | 0.06  | +      | 26                                 |    |
|                | 370            | ♂   |                            |  | 0.09  | +      | 20                                 |    |
|                | 390            | ♂   |                            | 1.0  | 0.03  | +      | 50                                 |    |
| September 2..  | 520            | ♀   |                            | 15 per cent solu-<br>tion                                    | 1.0   | 0.02   | +                                  | 5  |
|                | 500            | ♀   |                            |  |   | 0.02   | +                                  | 5  |
|                | 430            | ♂   | 0.02                       |  |   | +      | 12                                 |    |
|                | 440            | ♀   | 0.02                       |  |   | —      |                                    |    |
|                | 400            | ♀   | 0.02                       |  |   | —      |                                    |    |
|                | 390            | ♂   | 0.03                       |  |   | —      |                                    |    |
|                | 410            | ♀   | 0.05                       |  |   | +      | 7                                  |    |
| July 11.....   | 390            | ♂   | 0.5                        |  |   | 0.03   | —                                  |    |
|                | 320            | ♂   | 1.0                        |  |   | 0.03   | —                                  |    |
|                | 350            | ♂   | 1.5                        |  |   | 0.03   | —                                  |    |
|                | 350            | ♀   | 1.5                        |  |   | 0.045  | —                                  |    |
|                | 350            | ♂   | 1.5                        |  |   | 0.06   | —                                  |    |
|                | 370            | ♀   | 1.5                        |  |   | 0.09   | +                                  | 20 |
|                | 360            | ♂   | 1.5                        |  |   | 0.175  | +                                  | 15 |
|                | 350            | ♂   |                            | 0.03   | +   | 50     |                                    |    |
|                | 320            | ♀   |                            | 0.03   | +   | 6      |                                    |    |
|                | 470            |     |                            | 0.03   | +   | 32     |                                    |    |
|                | September 2..  | 520 | ♀                          | 1.0  | 1.0   | 0.02   | +                                  | 5  |
|                |                | 500 | ♀                          |  |   | 0.02   | +                                  | 5  |
| 470            |                | ♂   | 0.02                       |  |   | +      | 55                                 |    |
| 340            |                | ♂   | 0.5                        |  |   | 0.02   | —                                  |    |
| 380            |                | ♂   | 1.5                        |  |   | 0.06   | —                                  |    |
| 370            |                | ♀   | 1.5                        |  |   | 0.1    | —                                  |    |
| 490            |                | ♀   | 1.5                        |  |   | 0.15   | +                                  | 14 |



TABLE 2—Continued

| DATE<br>(1918)  | BODY-<br>WEIGHT | SEX | AMOUNT OF GLUCOSE<br>MIXED | AMOUNT<br>OF NaCl<br>SOLUTION<br>(0.85 PER<br>CENT)<br>MIXED | AMOUNT<br>OF LUNG<br>EXTRACT<br>PER 100<br>GRAMS OF<br>BODY<br>WEIGHT | RESULT | TIME FROM<br>INJECTION<br>TO DEATH |
|-----------------|-----------------|-----|----------------------------|--|---|--------|------------------------------------|
|                 | <i>grams</i>    |     | <i>cc.</i>                 | <i>cc.</i>   | <i>cc.</i>  |        | <i>minutes</i>                     |
|                 |                 |     | 42 per cent solu-<br>tion  |  |   |        |                                    |
| August 17 . . . | 830             | ♀   |                            |  | 0.1   | +      | 18                                 |
|                 | 500             | ♀   |                            |  | 0.1   | +      | 13                                 |
|                 | 580             | ♀   |                            |  | 0.1   | +      | 38                                 |
|                 | 560             | ♀   | 0.5                        |  | 0.1   | +      | 43                                 |
|                 | 500             | ♀   |                            | 0.5  | 0.1   | +      | 15                                 |
|                 | 560             | ♀   | 1.0                        |  | 0.1   | +      | 19                                 |
|                 | 680             | ♀   | 1.5                        |  | 0.1   | +      | 60                                 |
|                 | 370             | ♀   |                            | 1.5  | 0.1   | +      | 20                                 |
|                 | 590             | ♀   | 2.0                        |  | 0.1   | —      |                                    |
|                 | 570             | ♀   | 2.5                        |  | 0.1   | —      |                                    |
|                 | 580             | ♀   |                            | 2.5  | 0.1   | +      | 40                                 |
|                 | 520             | ♂   | 3.0                        |  | 0.1   | —      |                                    |
|                 | 500             | ♂   |                            | 3.0  | 0.1   | +      | 13                                 |
|                 | 520             | ♀   |                            |  | 0.02  | +      | 5                                  |
|                 | 500             | ♀   |                            |  | 0.02  | +      | 5                                  |
| September 2 . . | 460             | ♀   | 0.5                        |  | 0.02  | —      |                                    |
|                 | 300             | ♂   | 1.0                        |  | 0.02  | —      |                                    |
|                 | 380             | ♂   | 0.5                        |  | 0.05  | +      | 10                                 |
|                 | 550             | ♂   | 1.5                        |  | 0.02  | —      |                                    |
|                 | 330             | ♀   | 1.5                        |  | 0.08  | —      |                                    |
|                 | 260             | ♀   | 1.5                        |  | 0.1   | —      |                                    |
|                 | 400             | ♂   | 1.5                        |  | 0.1   | —      |                                    |
|                 | 420             | ♀   | 1.5                        |  | 0.12  | +      | 7                                  |
|                 | 380             | ♂   | 1.5                        |  | 0.12  | —      |                                    |
|                 | 490             | ♀   |                            |  | 0.05  | +      | 6                                  |
| September 11 .  | 460             | ♂   |                            |  | 0.05  | +      | 55                                 |
|                 | 550             | ♂   | 0.5                        |  | 0.05  | +      | 8                                  |
|                 | 640             | ♂   | 1.0                        |  | 0.05  | +      | 18                                 |
|                 | 580             | ♂   | 1.0                        |  | 0.05  | —      |                                    |
|                 | 490             | ♀   | 1.5                        |  | 0.05  | —      |                                    |
|                 | 600             | ♀   | 1.5                        |  | 0.1   | —      |                                    |
|                 | 540             | ♀   | 1.5                        |  | 0.1   | +      | 17                                 |
|                 | 500             | ♂   | 1.5                        |  | 0.15  | +      | 28                                 |
|                 |                 |     |                            |  |   |        |                                    |

TABLE 2—*Concluded*

| DATE<br>(1918) | BODY<br>WEIGHT | SEX | AMOUNT OF GLUCOSE<br>MIXED | AMOUNT<br>OF NaCl<br>SOLUTION<br>(0.85 PER<br>CENT)<br>MIXED | AMOUNT<br>OF LUNG<br>EXTRACT<br>PER 100<br>GRAMS OF<br>BODY<br>WEIGHT | RESULT | TIME FROM<br>INJECTION<br>TO DEATH |
|----------------|----------------|-----|----------------------------|--|---|--------|------------------------------------|
|                | <i>grams</i>   |     | <i>cc.</i>                 | <i>cc.</i>   | <i>cc.</i>  |        | <i>minutes</i>                     |
| September 14.  | 360            | ♂   |                            |  | 0.15  | +      | 11                                 |
|                | 420            | ♀   |                            |  | 0.15  | +      | 7                                  |
|                | 430            | ♀   | 0.5                        |  | 0.15  | +      | 10                                 |
|                | 540            | ♂   | 1.0                        |  | 0.15  | —      |                                    |
|                | 420            | ♀   | 1.0                        |  | 0.15  | —      |                                    |
|                | 500            | ♂   | 1.5                        |  | 0.15  | —      |                                    |
|                | 500            | ♂   | 1.5                        |  | 0.15  | —      |                                    |
|                | 520            | ♀   | 1.5                        |  | 0.25  | —      |                                    |
|                | 370            | ♂   | 1.5                        |  | 0.3   | +      | 24                                 |
|                | 380            | ♀   | 1.5                        |  | 0.3   | —      |                                    |
| September 17.  | 420            | ♂   |                            |  | 0.1   | +      | 40                                 |
|                | 380            | ♂   |                            |  | 0.1   | +      | 55                                 |
|                | 410            | ♀   | 0.5                        |  | 0.1   | +      | 23                                 |
|                | 390            | ♂   | 1.0                        |  | 0.1   | +      | 16                                 |
|                | 400            | ♂   | 1.5                        |  | 0.1   | —      |                                    |
|                | 410            | ♂   | 1.5                        |  | 0.2   | —      |                                    |
|                | 400            | ♂   | 1.5                        |  | 0.2   | —      |                                    |
|                | 420            | ♀   | 1.5                        |  | 0.3   | +      | 14                                 |

The results obtained in the above experiments may be summarized as following:

1. One cubic centimeter of 10 per cent solution of glucose is effective to protect guinea pigs against the minimal lethal dose of the beef lung extract and 1.5 cc. of the same solution protects against 1.5 times the lethal dose.

2. Five-tenths cubic centimeter of 15 per cent solution is also effective against the minimal lethal dose and 1.5 cc. against 2 to 3 times the lethal dose.

3. Five-tenths to one cubic centimeter of 42 per cent solution is effective against the minimal lethal dose and 1.5 cc. against 1.5 to 5.0 times the minimal lethal dose.

Therefore it is certain that glucose is effective in protecting guinea pigs against the beef lung extract, but the nature of such action is still obscure.

TABLE 3

*The influence of adrenalin upon the toxicity of the beef lung extract for guinea pigs*

| DATE<br>(1918)    | BODY<br>WEIGHT | SEX | AMOUNT OF<br>ADRENALIN<br>INJECTED | AMOUNT OF<br>EXTRACT IN-<br>JECTED PER<br>100 GRAMS<br>OF BODY<br>WEIGHT | RESULT | TIME FROM<br>INJECTION<br>TO DEATH |
|-------------------|----------------|-----|------------------------------------|--|--------|------------------------------------|
|                   | grams          |     | cc.                                | cc.  |        | minutes                            |
| August 12.....    | 420            | ♂   |                                    | 0.05   | —      |                                    |
|                   | 420            | ♂   |                                    | 0.05   | +      | 10                                 |
|                   | 550            | ♀   |                                    | 0.1  | +      | 45                                 |
|                   | 420            | ♂   |                                    | 0.1  | +      | 15                                 |
|                   | 390            | ♂   | 0.3                                | 0.1  | —      |                                    |
|                   | 570            | ♂   | 0.2                                | 0.1  | —      |                                    |
|                   | 380            | ♂   |                                    | 0.15   | +      | 55                                 |
|                   | 440            | ♀   |                                    | 0.15   | +      | 5                                  |
|                   | 450            | ♂   | 0.2                                | 0.15   | —      |                                    |
|                   | 560            | ♂   | 0.2                                | 0.15   | —      |                                    |
|                   | 490            | ♀   |                                    | 0.2  | +      | 9                                  |
|                   | 450            | ♀   | 0.3                                | 0.2  | +      | 30                                 |
|                   | 570            | ♀   | 0.3                                | 0.2  | +      | 24                                 |
|                   | 360            | ♀   | 0.2                                | 0.2  | +      | 20                                 |
|                   | 470            | ♂   | 0.2                                | 0.2  | +      | 16                                 |
| September 2.....  | 520            | ♀   |                                    | 0.02   | +      | 5                                  |
|                   | 500            | ♀   |                                    | 0.02   | +      | 5                                  |
|                   | 300            | ♀   | 0.2                                | 0.02   | —      |                                    |
|                   | 450            | ♀   | 0.2                                | 0.02   | —      |                                    |
|                   | 350            | ♂   | 0.2                                | 0.03   | —      |                                    |
|                   | 390            | ♂   | 0.2                                | 0.04   | +      | 8                                  |
|                   | 330            | ♂   | 0.2                                | 0.04   | —      |                                    |
|                   | 340            | ♀   | 0.2                                | 0.05   | +      | 25                                 |
|                   | 390            | ♂   | 0.2                                | 0.05   | —      |                                    |
|                   | 340            | ♀   | 0.2                                | 0.06   | +      | 20                                 |
|                   | 390            | ♂   | 0.2                                | 0.06   | —      |                                    |
|                   | 490            | ♀   |                                    | 0.05   | +      | 6                                  |
| September 11..... | 460            | ♂   |                                    | 0.05   | +      | 55                                 |
|                   | 540            | ♀   | 0.2                                | 0.05   | —      |                                    |
|                   | 400            | ♂   | 0.2                                | 0.1  | +      | 15                                 |
|                   | 390            | ♂   | 0.2                                | 0.1  | +      | 27                                 |
|                   | 580            | ♀   | 0.2                                | 0.1  | —      |                                    |
|                   | 350            | ♀   | 0.2                                | 0.15   | +      | 8                                  |
| September 14..... | 360            | ♂   |                                    | 0.15   | +      | 3                                  |
|                   | 420            | ♀   |                                    | 0.15   | +      | 7                                  |
|                   | 520            | ♀   | 0.2                                | 0.15   | +      | 5                                  |
|                   | 480            | ♂   | 0.2                                | 0.15   | —      |                                    |

TABLE 3—*Concluded*

| DATE<br>(1918)    | BODY<br>WEIGHT | SEX | AMOUNT OF<br>ADRENALIN<br>INJECTED | AMOUNT OF<br>EXTRACT IN-<br>JECTED PER<br>100 GRAMS<br>OF BODY<br>WEIGHT | RESULT | TIME FROM<br>INJECTION<br>TO DEATH |
|-------------------|----------------|-----|------------------------------------|--|--------|------------------------------------|
|                   | <i>grams</i>   |     | <i>cc.</i>                         | <i>cc.</i>   |        | <i>minutes</i>                     |
| September 17..... | 410            | ♀   | 0.3                                | 0.15   | —      |                                    |
|                   | 370            | ♀   | 0.2                                | 0.25   | +      | 39                                 |
|                   | 330            | ♀   | 0.2                                | 0.25   | +      | 17                                 |
|                   | 470            | ♂   | 0.3                                | 0.3  | +      | 4                                  |
|                   | 390            | ♀   | 0.2                                | 0.3  | —      |                                    |
| September 17..... | 420            | ♂   |                                    | 0.1  | +      | 40                                 |
|                   | 380            | ♂   |                                    | 0.1  | +      | 55                                 |
|                   | 390            | ♀   | 0.2                                | 0.1  | —      |                                    |
|                   | 420            | ♂   | 0.2                                | 0.1  | +      | 40                                 |
|                   | 440            | ♀   | 0.2                                | 0.15   | —      |                                    |
|                   | 430            | ♂   | 0.2                                | 0.15   | +      | 21                                 |
|                   | 440            | ♂   | 0.2                                | 0.15   | +      | 12                                 |
|                   | 430            | ♀   | 0.2                                | 0.2  | +      | 28                                 |

#### ON THE INFLUENCE OF ADRENALIN UPON THE TOXIC EFFECT OF THE EXTRACT

There are many clinical and experimental publications on the toxin-neutralizing power of adrenalin, but all these reports do not harmonize and the nature of the neutralizing action is yet unknown. The following experiments have been made to determine the biological influence of adrenalin on the extract of the beef lung.

After the hypodermic injection of 0.2 to 0.3 cc. of adrenalin (adrenalin chloride, 1: 1000, Sankyo and Co.) the extract of beef lung was immediately injected into the external jugular vein of guinea pigs.

From table 3 it is to be seen that the hypodermic injection of adrenalin (0.2 to 0.3) is effective to protect guinea pigs against 1 to 1.5 times the minimal lethal dose of the beef lung extract.

#### ON THE INFLUENCE OF THE INTRAVENOUS INJECTION OF THE LUNG EXTRACT UPON THE SUGAR CONTENT OF THE BLOOD

After studying the neutralizing and protective effects of glucose and adrenalin against the lung extract, the influence of the toxin upon the blood sugar was taken up for investigation. It is well known that

hyperglycemia is caused by injection of various toxins. The lung extract of guinea pigs was injected intravenously into rabbits and the blood sugar was determined by Bang's micromethod. In this series of experiments, rabbits were mostly used and special precautions were taken to avoid other influencing factors.

TABLE 4

*The influence of the lung extract of guinea pigs on the sugar content of the blood of rabbits*

| BODY WEIGHT  | SEX | AMOUNT OF EXTRACT INJECTED | BLOOD SUGAR CONTENT (PER CENT) |                       |       |                |       |                |       |       |       |       |
|--------------|-----|----------------------------|--------------------------------|-----------------------|-------|----------------|-------|----------------|-------|-------|-------|-------|
|              |     |                            | Before injection               | Hours after injection |       |                |       |                |       |       |       |       |
|              |     |                            |                                | $\frac{1}{2}$         | 1     | $1\frac{1}{2}$ | 2     | $2\frac{1}{2}$ | 3     | 4     | 5     | 6     |
| <i>grams</i> |     | <i>cc.</i>                 |                                |                       |       |                |       |                |       |       |       |       |
| 2740         | ♂   | 1.5                        | 0.132                          | 0.127                 | 0.137 | 0.153          | 0.155 | 0.150          | 0.182 | 0.150 | 0.149 |       |
| 1790         | ♂   | 1.5                        | 0.120                          | 0.113                 | 0.114 | 0.104          | 0.103 | 0.099          | 0.116 | 0.122 | 0.119 |       |
| 1680         | ♀   | 2.0                        | 0.095                          | 0.084                 | 0.081 | 0.101          | 0.111 | 0.110          | 0.118 | 0.114 | 0.101 |       |
| 2370         | ♂   | 2.0                        | 0.104                          | 0.105                 | 0.111 | 0.112          | 0.108 | 0.118          | 0.131 | 0.103 | 0.102 | 0.104 |
| 2560         | ♀   | 1.5                        | 0.118                          | 0.100                 | 0.110 | 0.115          | 0.128 | 0.119          | 0.135 | 0.121 | 0.119 |       |

This table shows that the injection of the lung extract causes a slight increase in the blood sugar.

## SUMMARY

1. Intravenous injection of beef lung extract in guinea pigs invariably causes dyspnoea and, in a majority of cases, convulsions as well.

2. The minimal lethal dose of the beef lung extract for guinea pigs on intravenous inoculation varied from 0.02 to 0.15 cc. per 100 grams of body weight.

3. Glucose destroys the toxicity of lung extracts. Thus 1.0 cc. of 10 per cent glucose mixed with the minimal lethal dose of lung extract renders the latter inert. Additional figures are given in the text.

4. Hypodermic injection of adrenalin immediately before an intravenous lethal dose of lung extract was protective in effect.

5. Intravenous injection of lung extract causes a slight increase in the blood sugar of rabbits.

I take this opportunity to express my gratitude to Prof. Dr. S. Mita for his suggestions, advice and criticism in carrying out this investigation.



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## VITAL STAINING AND ACIDOSIS

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### INTRODUCTION

The vital staining method by injection of lithium carmine solution into the ear vein of living animals has recently been applied to investigations in the field of medicine and biology. But the chemical reaction between the stain and the living cells is as yet obscure. Many authors assume some specific granula in the protoplasm of the living cells which serve to take up the stain. Kiyono (1), (2) assumes their increase in the supravital state from the fact that carmine granula taken up by surviving cells are always more in number and finer in size. Though nearly all living cells take carmine stains, the epithelium of the bile-duct is never found to be stained because, presumably, of its alkaline reaction, while cells of brain, plexus chorioideus ventriculi quarti and the intestinal mucous membrane take the stains in a trace and in a state of the finest granula, again presumably on account of their rather alkaline reaction. We know also that the carmine stains are quite toxic to the living cells and because of this an ideal staining requires one administration a day in a not very toxic dose, at least for several days. From these data I would like to conceive that the carmine granula are not due to staining of the specific intracellular granula, but are due rather to a physical and physico-chemical phenomenon, which takes place as a consequence of acidosis in the organism caused by the carmine injection.

This research was begun for the purpose of testing this hypothesis in October, 1917, and concluded in April, 1918, at the Institute for Forensic Medicine of the Tokyo Imperial University. It was presented before the Pathological Section of the Fifth Japanese Medical Congress held in April, 1918, in Tokyo. In connection with this report I acknowledge my pleasant obligation to express gratitude to Prof. Dr. K. Katayama and Prof. Dr. S. Mita for their kind direction and helpful advice.

## EXPERIMENTAL

First I demonstrated the precipitation of carmine granula from the lithium carmine in a very dilute solution of hydrochloric acid in vitro, secondly the acidosis in the blood of guinea pigs in which the stains were intraperitoneally administered, and thirdly I realized a more conspicuous staining in animals in which acidosis was previously called forth by various manipulations.

The method carried out and the results obtained were as follows:

1. *Precipitation of carmine granula from the lithium carmine solution made acid by the addition of a very small amount of hydrochloric acid.* I dissolved 5 grams of the purest carmine (Grübler) in a saturated solution of lithium carbonicum. The reaction of the solution obtained was strongly alkaline, that is, it blued litmus paper sharply. There was never found microscopically any visible granula in the solution after filtration through gauze. I put it into a test tube, diluted it with

TABLE I  
*Plasma bicarbonate of normal guinea pigs*

|  | GUINEA PIG |        |        | AVERAGE |
|--|------------|--------|--------|---------|
|  | 6          | 8      | 9      |         |
| Body weight (grams).....                   | 510        | 510    | 544    |         |
| CO <sub>2</sub> (cc. in 1 cc. plasma)..... | 0.3806     | 0.4373 | 0.4082 | 0.4087  |

aqua distillata or blood plasma of normal rabbits and microscopically found as before no visible carmine granula in it. But as soon as I added some drops of 0.1 per cent solution of hydrochloric acid the color of the carmine red solution turned a little pale, and in a sample of it many red granula became microscopically visible. After 24 hours the solution was separated in two different parts: the greater upper one consisting of clear colorless watery fluid and the smaller lower of a red sediment. I observed the similar precipitation also by addition of various electrolytes, e.g., ammonium sulphate, calcium chloride, silver chloride, etc., and also of various acids, but it did not happen when sodium bicarbonate, sodium chloride, etc., were added; in the case of Ringer's solution it became purplish without, however, any precipitation. The lithium carmine solution was dialysable through the thimble (Carl Schleicher and Schüll) extraordinarily slowly.

It is evident from these in-vitro experiments that carmine is capable of dissolving in alkaline lithium carbonicum solution in a state of col-

loidal solution, but is liable to precipitation in a slightly acidified solution or a slight excess of hydrogen ion concentration on the one hand and also in the presence of electrolytes of an easily dissociable sort on the other hand.

2. *Acidosis in the blood plasma of guinea pigs stained vitally with lithium carmine.* I injected intraperitoneally 1 or 2 cc. of 5 per cent lithium carmine solution, after sterilization and filtration through gauze, into guinea pigs once a day for more than ten days. I collected

TABLE 2

*Body weight loss of guinea pigs in the course of vital staining with lithium carmine solution*

| DAYS OF<br>EXPERIMENT | GUINEA PIG  |             |             | AMOUNT OF<br>CARMINE INJECTED |
|-----------------------|-------------|-------------|-------------|-------------------------------|
|                       | 3           | 4           | 5           |                               |
|                       | Body weight | Body weight | Body weight |                               |
|                       | grams       | grams       | grams       | cc.                           |
| 1                     | 590         | 435         | 550         | 1.0                           |
| 2                     | 570         | 435         | 560         | 1.0                           |
| 3                     | 590         | 435         | 590         | 1.0                           |
| 4                     | 610         | 400         | 615         | 1.0                           |
| 5                     |             |             |             |                               |
| 6                     | 620         | 428         | 600         | 1.0                           |
| 7                     | 636         | 416*        | 620         | 2.0                           |
| 8                     | 600         | 400         | 560         | 2.0                           |
| 9                     | 530         | 410         | 530         | 2.0                           |
| 10                    | 500         | 380         | 500         | 2.0                           |
| 11                    | 480         | 360         | 476         | 2.0                           |
| 12                    |             |             |             |                               |
| 13                    | 420†        |             |             |                               |
| 14                    |             |             | 390†        |                               |

\* Blood drawn.

† Blood drawn and killed.

the arterial blood from the carotid by help of a cannula into a paraffined centrifuge tube containing a layer of paraffin oil and provided with potassium oxalate to make about 0.5 per cent the weight of the blood drawn, under precautions to prevent its free contact with air and to avoid any agitation. The blood thus drawn was centrifuged immediately and 1 cc. of the plasma was subjected to Van Slyke and Cullen's determination of bicarbonate. The determination was carried out two or three times on the one and the same sample and their average figure

was adopted as a result. As two or more bleedings reduce the amount of plasma bicarbonate, as I have previously shown in fasting rabbits (4), the first blood sample only was used to the present experiments.

a. *Plasma bicarbonate of normal guinea pigs.* As seen from the table 1, in guinea pigs the plasma bicarbonate was determined at 40.87 volume per cent, i.e., much less than about 60, the value in man, rabbits and many other animals. It was, I think, because of their far more vivacious motion than that of rabbits, etc., before being attached to the fastening apparatus.

b. *Body weight loss of guinea pigs in the course of vital staining with lithium carmine.* As shown in table 2, the intraperitoneal administration of 1 cc. lithium carmine to three guinea pigs of 435 to 590 grams, i.e., of 0.19 cc. per 100 grams seems not to affect their health, but that of 2 cc. to the animals of 416 to 636 grams, i.e., of 0.358 cc. per 100 grams, markedly reduces the body weight.

TABLE 3  
*Plasma bicarbonate of guinea pigs after vital staining*

|  | GUINEA PIG |        |        |
|--|------------|--------|--------|
|  | 3          | 4      | 5      |
| Day of blood drawing.....                        | 13th       | 7th    | 14th   |
| CO <sub>2</sub> (cubic centimeter).....          | 0.2354     | 0.3109 | 0.1573 |
| CO <sub>2</sub> (milligram in 1 cc. plasma)..... | 0.4625     | 0.6066 | 0.3082 |

Therefore it may be said that 5 per cent lithium carmine has a toxic action when more than 0.35 cc. per 100 grams is intraperitoneally administered to guinea pigs.

c. *Plasma bicarbonate of guinea pigs after vital staining with 5 per cent lithium carmine solution.* These results are presented in table 3. Taking 40.87 per cent as the normal value for the arterial plasma bicarbonate, it will be seen that the treatment with lithium carmine solution has a very decided effect. No. 4, on the seventh day of the experiment, with an insignificant loss in body weight, gave 31 per cent plasma bicarbonate. No. 3, on the thirteenth day of the experiment, two days after the injections had been stopped, showed a loss in body weight of 34 per cent and a plasma bicarbonate of only 23.54 per cent. In no 5 the acidosis was so far advanced on the fourteenth day of the experiment, i.e., three days after the final carmine injection, that the plasma bicarbonate amounted to only 15.73 per cent. At this time the animal had lost 37 per cent of its body weight.



TABLE 4  
*Preinjection of alkali or acid before carmine injection*

|                    | DATE                    | GROUP                      |                            |                            |  |  |  |                      |                      |  |
|--------------------|-------------------------|----------------------------|----------------------------|----------------------------|--|--|--|----------------------|----------------------|--|
|                    |                         | I                          |                            |                            | II   |  |  | III                  |                      |  |
|                    |                         | Rabbit 1                   | Rabbit 6                   | Rabbit 7                   | Rabbit 3                                   | Rabbit 4                                   | Rabbit 8                                   | Rabbit 2             | Rabbit 5             |  |
| Body weight. . .   | 1                       | 1986                       | 2040                       | 1528                       | 1926                                       | 1898                                       | 1830                                       | 1558                 | 2418                 |  |
|                    | 2                       | 2144                       | 2170                       |                            | 1868                                       | 1896                                       |  | 1530                 | 2660                 |  |
|                    | 3                       | 2036                       | 2100                       |                            | 1900                                       | 1756                                       |  | 1490                 | 2466                 |  |
|                    | 4                       | 2012                       | 2156                       |                            | 1818                                       | 1800                                       |  | 1532                 | 2480                 |  |
|                    | 5                       |                            |                            |                            |  | 1808                                       |  |                      | 2588                 |  |
| Preinjection       | 1                       | $\frac{N}{10}$ HCl, 20 cc. | $\frac{N}{10}$ HCl, 10 cc. | $\frac{N}{30}$ HCl, 45 cc. | $\frac{N}{30}$ NaHCO <sub>3</sub> , 10 cc. | $\frac{N}{10}$ NaHCO <sub>3</sub> , 19 cc. | $\frac{N}{30}$ NaHCO <sub>3</sub> , 54 cc. | Without preinjection | Without preinjection |  |
|                    | 2                       | 10 cc.                     | 20 cc.                     |                            | 10 cc.                                     | 8 cc.                                      |  |                      |                      |  |
|                    | 4                       |                            | $\frac{N}{30}$ HCl, 40 cc. |                            |  | $\frac{N}{30}$ NaHCO <sub>3</sub> , 40 cc. |  |                      |                      |  |
|                    |                         |                            |                            |                            |  |  |  |                      |                      |  |
| Carmine Injection. | 1                       |                            |                            | 8.0 cc.                    | 5.0 cc.                                    | 5.0 cc.                                    | 9.0 cc.                                    | 5.0                  | 5.0 cc.              |  |
|                    | 3                       | 5.0 cc.                    | 5.0 cc.                    |                            |  | 5.0 cc.                                    |  |                      | 6.0 cc.              |  |
|                    | 4                       |                            | 8.0 cc.                    |                            |  |  |  |                      |                      |  |
|                    | Time after preinjection |                            | 30'                        | 50'                        |  | 30'  | 40'  |                      |                      |  |

|                     |             |                      |   |   |           |  |                          |                                |
|---------------------|-------------|----------------------|---|---|-----------|--|--------------------------|--------------------------------|
| Fats.....           | 1           | Killed               | Immediately<br>after<br>staining<br>dead with<br>cramps | About 1.5<br>hours<br>after<br>staining<br>killed | Killed    | About 1<br>hour<br>after<br>staining<br>killed | Killed                   | Killed                         |
|                     | 4           |                      |   |   |           |  |                          |                                |
|                     | 5           |                      |   |   |           |  |                          |                                |
| Urine in<br>bladder | Reaction    | Slightly<br>alkaline | Amphoteric  | Slightly<br>acid                                  | Acid      | Slightly<br>acid                               | Alkaline                 | Amphoteric                     |
|                     | Color, etc. | Red<br>turbid        | Slightly<br>turbid                                      | Carmine<br>red                                    | Red clear | Slightly<br>turbid<br>carmine<br>red           | Carmine<br>red<br>turbid | Reddish                        |
|                     |             |                      |   |   |           |  |                          | Slightly<br>alkaline           |
|                     |             |                      |   |   |           |  |                          | Reddish<br>intensely<br>turbid |

Since the intraperitoneal administration of 0.19 cc. of 5 per cent lithium carmine per 100 grams body weight for six days gave incomplete vital staining, the dose was increased to 0.358 cc. per 100 grams, and continued for five days more. This yielded a satisfactory stain but left the animal moribund in extreme acidosis and loss of body weight.

3. *Vital staining of animals previously made acidotic.* In order to call forth acidosis, I injected a slightly acid or a slightly alkaline solution into the ear vein or into the peritoneal cavity of a rabbit or a mouse. It was possible then to vitally stain the animal to a very high degree with a single injection of the carmine solution.

a. *Vital staining of rabbits after preinjection of alkali or acid.* To this series of experiments, 8 rabbits were used, classifying them into 3 groups; to the first group a tenth or thirtieth normal solution of hydrochloric acid was administered into the ear veins, to the second a tenth or thirtieth normal solution of sodium bicarbonate, and the third remained without any preinjection.

As the blood was very liable to coagulate in the presence of acid, I could not easily inject a sufficient amount of the tenth normal solution. A concentration of a thirtieth normal solution was relatively easily administered. It was the same in the case of alkali. After these preinjections 5 per cent lithium carmine was administered intravenously in intervals of from 30 minutes to 24 hours. The animals thus stained vitally were killed by means of bleeding from carotid at various times after the final injection. At autopsy nearly all the glandular organs were carmine red, but the adrenal was almost always yellowish. In bladder the urine was very interesting: In the urine of No. 7, which was killed about 2 hours after the preinjection of 45 cc.  $\frac{N}{10}$  HCl and an hour after the intravenous administration of 8 cc. lithium carmine, carmine granula and bladder epithelia with carmine red nuclei were found abundantly under the microscope. The urine was slightly turbid, slightly acid and of carmine red color. The urine of no. 8, which was killed also about 2 hours after the preinjection of 54 cc.  $\frac{N}{30}$  NaHCO<sub>3</sub> and an hour after the intravenous administration of 9 cc. lithium carmine, was also carmine red, turbid and of alkaline reaction, and contained no carmine granula nor cells with stained nuclei. The urine of no. 4 which was killed about 24 hours after the final preinjection of alkali and carmine administration, was carmine red, turbid and slightly

acid. The urine of no. 6, which died with cramps immediately after the carmine injection to the amount of 8 cc. and 30 minutes after the third preinjection of a thirtieth normal solution of hydrochloric acid amounting to 40 cc., was slightly turbid, slightly red and of amphoteric reaction. The urines of those which were killed about 40 hours after the second preinjection of alkali or acid and about 20 hours after the carmine injection, gave, in general, a reaction the opposite of that of the preinjected solution. The urines of those which were not preinjected with acid or alkali before the carmine staining, remained slightly alkaline or amphoteric. Generally speaking, in a short time after preinjection of acid or alkali, the urine is of nearly normal reaction, but 24 to 40 hours after the preinjection its reaction becomes quite the opposite of the preinjected solution in spite of the alkaline reaction of the carmine which was always administered afterwards.

TABLE 5

*Distribution of carmine in body organs of the preinjected rabbits after vital staining*

|                             | RABBIT |   |   |   |   |   |   |   |
|-----------------------------|--------|---|---|---|---|---|---|---|
|                             | 1      | 6 | 7 | 3 | 4 | 8 | 2 | 5 |
| Distribution of carmine in: |        |   |   |   |   |   |   |   |
| Liver.....                  | ±      | ± | — | ± | ± | ± | ± | ± |
| Kidney.....                 | —      | — | + | — | — | + | — | — |
| Spleen.....                 | —      | — | — | — | — | — | — | — |
| Brain.....                  | —      | — | — | — | — | — | — | — |
| Heart.....                  | —      | — | — | — | — | — | — | — |

This fact is very interesting in connection with explanation of carmine-acidosis, because the repeated injections of alkaline carmine solution may of itself cause an acidosis in the experimental animals.

At autopsy there were no special changes aside from the carmine staining of the internal organs. But on histological examination of the tissues I found nearly always cloudy swelling, coagulation, vascularization and fatty degeneration of different degree in the liver, kidney, spleen, and some other organs, without any conspicuous difference under the three groups.

With regard to the distribution of carmine granula in various organs also, as shown in table 5, I cannot find any special difference under the three groups. In the kidney of no. 6 which died immediately after the carmine injection, no carmine granula were found, but in that of no. 7 and 8 which were killed in 1 or 1.5 hours after the vital staining, the

TABLE 6  
*Vital staining of mice after preinjection of acid or alkali*

| MOUSE<br>NUM-<br>BER | CARBINATION          |        | STAIN  | TIME OF DECAPITATION AFTER<br>STAINING | DISTRIBUTION OF CARMINE |        |       |        |
|----------------------|----------------------|--------|--------|--|-------------------------|--------|-------|--------|
|                      | Kind                 | Amount |        |  | Blood                   | Spleen | Liver | Kidney |
|                      |                      | cc.    |        |  |                         |        |       |        |
| 3                    | N HCl                | p. 0.5 | v. 0.1 | 3 hrs.                                 | —                       |        |       |        |
| 4                    | N HCl                | p. 0.5 | v. 0.1 | 3 hrs.                                 | Trace                   |        |       |        |
| 6                    | N HCl                | p. 0.5 | v. 0.2 | 1.5 hrs.                               | Trace                   |        |       |        |
| 7                    | N HCl                | p. 0.5 | v. 0.2 | 1.5 hrs.                               | —                       |        |       |        |
| 19                   | N HCl                | p. 0.5 | p. 0.5 | 2 hrs.                                 | Trace                   |        |       |        |
| 32                   | N HCl                | p. 0.5 | p. 0.5 | 2 hrs.                                 | —                       |        |       |        |
| 33                   | N HCl                | p. 0.5 | p. 0.5 | 2 hrs.                                 | —                       | ±      | Trace | —      |
| 43                   | N HCl                | p. 0.5 | p. 0.5 | 20'                                    | —                       | ±      | —     | ±      |
| 44                   | N HCl                | p. 0.5 | v. 0.1 | 20'                                    | —                       | —      | —     | —      |
| 45                   | N HCl                | p. 0.5 | v. 0.1 | 20'                                    | —                       | —      | —     | —      |
|                      |                      |        |        |  |                         |        |       |        |
| 1                    | N NaHCO <sub>3</sub> | p. 0.5 | v. 0.1 | 3 hrs.                                 | —                       |        |       |        |
| 2                    | N NaHCO <sub>3</sub> | p. 0.5 | v. 0.1 | 3 hrs.                                 | —                       |        |       |        |
| 8                    | N NaHCO <sub>3</sub> | p. 0.5 | v. 0.1 | 3 hrs.                                 | —                       |        |       |        |
| 9                    | N NaHCO <sub>3</sub> | p. 0.5 | v. 0.1 | 3 hrs.                                 | —                       |        |       |        |
| 26                   | N NaHCO <sub>3</sub> | p. 0.5 | p. 0.3 | 1.5 hrs.                               | Trace                   |        |       |        |
| 27                   | N NaHCO <sub>3</sub> | p. 0.3 | p. 0.3 | 1.5 hrs.                               | —                       |        |       |        |
| 29                   | N NaHCO <sub>3</sub> | p. 0.5 | p. 0.5 | 2 hrs.                                 | —                       |        |       |        |
| 30                   | N NaHCO <sub>3</sub> | p. 0.5 | p. 0.5 | 2 hrs.                                 | +                       | +      | ++    | +      |
| 31                   | N NaHCO <sub>3</sub> | p. 0.5 | p. 0.5 | 2 hrs.                                 | +                       | +      | ++    | +      |
|                      |                      |        |        |  |                         |        |       |        |
| 5                    | Without              |        | v. 0.5 | Dead on the spot                       | ++                      |        |       |        |
| 28                   | Without              |        | p. 0.3 | 1.5 hrs.                               | —                       | Trace  |       |        |
| 37                   | Without              |        | v. 0.2 | 2 hrs.                                 | —                       | —      |       |        |

N. B. The abbreviation v. indicates intravenous injection and p. intraperitoneal one.



staining was conspicuous; abundant in bases of epithelia of the tubuli contorti of no. 8 and in traces in lumina of urine-tubules of no. 7.

b. *Vital staining of mice after preinjection of acid or alkali.* Since rabbits require a large amount of carmine I resorted to the use of mice in order to spare the stains. Their weight was 10 to 15 grams. The preinjections were carried out to the amount of 0.5 cc. for the most part into the peritoneal cavity, and the stain was generally injected into the tail vein to the amount of 0.1 or 0.2 cc.; the intravenous administration of 0.3 cc. of the stain often killed the animals at once, but the intraperitoneal injection of 0.5 cc. was not so harmful.

In from 20 minutes to 3 hours after the vital staining the mice were killed by decapitation, and immediately smear preparations on cover-glasses were made from blood and liver on the one hand, and on the other hand pieces of mesentery were stretched out between two cover-glasses. These preparations were stained with hematoxylin only or combining eosin thereto, after their fixation in ether and alcohol. Also specimens of liver and kidney of most of the mice were imbedded in paraffin, cut and stained with hematoxylin and hematoxylin and eosin.

Microscopically I found no special difference between the two series, i.e., the animals treated with alkali and acid. Cloudy swelling, coagulation, pycnosis of the nucleus, karyorhexis or karyolysis were found in nearly all preparations. But as shown in table 6, the animals treated with alkali (nos. 29, 30, 31). took the stains conspicuously as compared with those treated throughout in the same manner except for the preinjection of acid (nos. 32, 33), in which only a trace of the stain was found. In the other animals even the carmine distribution did not differ. Those which were vitally stained by intravenous injection of less than 2 cc. carmine within 30 minutes of the preinjection nearly always gave negative results. Those which were vitally stained by intraperitoneal injection of 0.5 cc. carmine an hour and a half after the preinjection, on the other hand, gave positive results.

#### DISCUSSION

From a perusal of the above facts it may be readily seen that vital staining with lithium carmine produces an acidosis, and that if acidosis be established as a preliminary to the injection of lithium carmine, the staining is much more conspicuous than in animals not so treated.

The relationship between acidosis and vital staining is difficult to explain. The current conception of cloudy swelling (5) is helpful. In vitally stained tissue the association of the carmine granula and cell edema (cloudy swelling) is generally recognized. If the edema is

developed by a rise of intracellular osmotic pressure due to an abnormal splitting of the cellular proteins in the absence of an adequate oxygen supply the carmine solution may be carried in with the production of edema. It is conceivable then that the carmine granula are precipitated in the acid medium, thus completing the histological picture. This will explain the fact that carmine granula are found only in cells with easily permeable walls and which are exposed to a slow blood stream, e.g., the reticulo-endothelial cells or histiocytes, while these granula are never found in those cells which are constantly exposed to alkaline body fluids or are bathed in a rapid blood stream where water and carmine diffuse in hardly more rapidly than the crystalloids diffuse out, e.g., the cells of the brain, bile ducts and greater blood vessels.

We may account, similarly, for the ease in staining surviving cells as opposed to living cells. If coagulation developed as the result of increased hydrogen-ion concentration before the stain could enter, there would be no deposition of carmine granula. Further, even after the cell walls lose their semipermeable character, the nuclei, possessing a relatively high hydrogen-ion concentration and still permeable to the dye, will take the stain, which has already entered the cell, and make the nuclear figures distinct.

#### SUMMARY

Vital staining can not be accomplished by a single intravenous or intraperitoneal injection of lithium carmine in subtoxic doses in a healthy animal. If, however, an acidosis be first established, a single injection of the dye will give a satisfactory stain.

In animals which have been vitally stained, an actual decrease in plasma bicarbonate occurs.

Hence the conclusion is drawn that it is incorrect to predicate the existence of specific stain-taking substances or granula in the cells. Rather, vital staining with lithium carmine is due to the development of an acidosis which so alters the function of the body cells that the dye diffusing in is deposited in granula. This deposition corresponds to the precipitation from colloidal solution of the dye when the normally alkaline solution is made acid in vitro.

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## STUDIES IN SECONDARY TRAUMATIC SHOCK

### IV. THE BLOOD VOLUME CHANGES AND THE EFFECT OF GUM ACACIA ON THEIR DEVELOPMENT

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In our earlier shock studies the hemodynamic findings all pointed directly to a decreased effective blood volume as the only constant factor tending toward failure of the circulation. Attention has also been called by numerous other observers to the significance of the reduction in both the effective and absolute volumes in this condition (1).

The present experiments were designed to determine the rôle that the absolute volume of the blood plays in the reduction of the effective volume in shock, and to evaluate the possible modes by which such a reduction might be brought about. Three possibilities suggest themselves as to ways the blood volume may be reduced: 1, hemorrhage, external or into tissues; 2, concentration of the blood, i.e., by filtration of the plasma; and 3, stasis in a portion of the vascular bed.

Four forms of experimental shock were studied, namely, those produced by injections of massive doses of adrenalin; by clamping the vena cava above the liver for a period of three hours so that the general arterial pressure was 30 to 40 mm. of mercury; by clamping the abdominal aorta above the coeliac axis for a period of three hours so that the distal pressure was 30 mm. of mercury and by exposure and manipulation of the intestines. Dogs were used in all experiments.

External hemorrhage was absolutely excluded except for the small amounts of blood necessary for the samples. The blood pressure was determined by a small bore manometer connected directly to the artery. It was seldom necessary to "wash out" during the course of an experiment, so that the loss of very few cubic centimeters of blood was involved.

Samples were taken directly from a large artery through a clean dry cannula. The red blood cells were counted as an index to the

relative plasma loss and the results obtained, therefore, indicate the minimum loss of blood through filtration of plasma as any local retention of the erythrocytes would decrease the count. In the later experiments the hemoglobin changes were determined by means of the Dubosque colorimeter, the blood being diluted 1/100 with 0.03 per cent HCl, and the normal hemoglobin dilution being considered as 100 per cent.

In case blood is withdrawn from circulation either by hemorrhage into tissue or stasis in a portion of the capillary bed, such a reduction could be appreciated only by a direct determination of the blood volume. Accordingly such determinations were made by the method of Meek and Gasser (2). Determinations may be made by this method to an accuracy within 5 per cent. Four cubic centimeters per kilo of 20 per cent acacia were injected intravenously, and ten minutes after the injection a sample of blood was taken for the distillations. All determinations were done in duplicate. If stasis occurs it might be of a degree varying from a local retardation of the stream to an absolute removal from active circulation. As ten minutes were allowed between injecting and sampling, the acacia would be diluted by all the blood that passes through the general circulation in a period of ten minutes.

In this series the final shock determinations were made after the mean arterial pressure had fallen to 50 mm. mercury. This was the conventional level chosen as a criterion early in our experience with shock before we came to appreciate that the attributes of shock usually develop long before this level is reached.

#### ADRENALIN SHOCK

The first studies were made on adrenalin shock. The normal volumes were determined and then the volumes in shock (table 1). Every case showed a reduction in blood volume; in two cases this reduction amounted to one-third of the total blood, in another case the blood volume was reduced by half. The blood counts on the other hand indicated a relatively small concentration, a finding that occasioned some surprise in view of the large concentrations obtained by Lamson (3) and by Bainbridge and Trevan (4). This discrepancy in results was traced directly to the acacia injected for the determination of the normal blood volume. One experiment was, therefore, performed in confirmation of the above authors. Four injections of adrenalin were made as indicated in figure 1a; with each of the first three there occurred



concentrations of the blood amounting to 29, 32 and 45 per cent of the normal respectively. In the intervals of lower blood pressure between the first three injections the red cell count returned to a condition in the neighborhood of normal. Each succeeding injection produced a concentration greater than the preceding although the adrenalin was given in smaller doses. Each injection must therefore have left a residuum of damage which became such after the third injection that the plasma did not return to the vessels as before. It

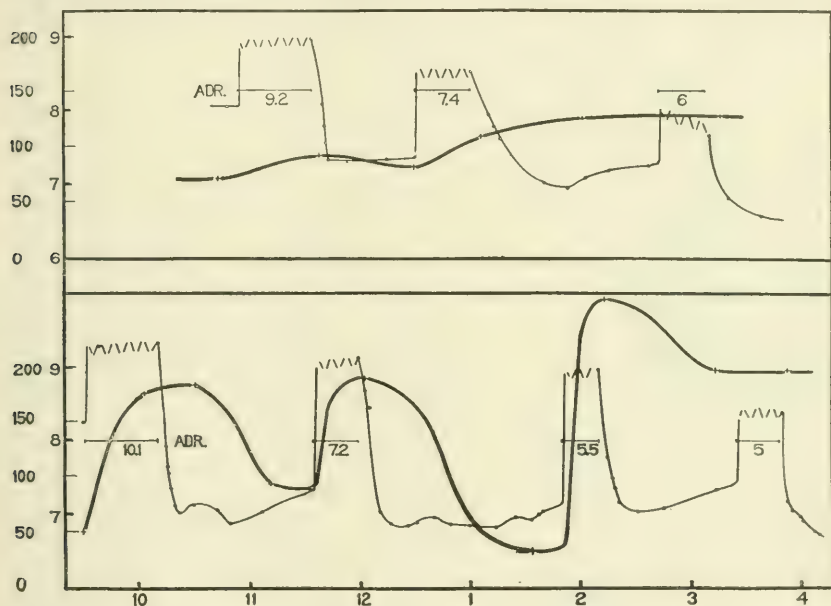


Fig. 1. (a) Lower: without acacia; (b) Upper: after the injection of 4 cc. per kilo of 20 per cent acacia. Light line: blood pressure in mm. Hg., indicated on the axis of ordinates. Heavy line: R. B. C. count in millions, indicated on the axis of ordinates. Axis of abscissas: time of day. Adrenalin injected at times indicated. Figures give numbers of cubic centimeters of 1/1000 injected.

is probable that only in this latter condition was the damage to the tissues sufficient to be spoken of as true shock.

A similar experiment was now performed in which 4 cc. per kilo of 20 per cent acacia were administered before adrenalin in comparable amounts was injected. The results (fig. 1b) show that the acacia not only has a marked effect on the final concentration attained but that it has an even more striking effect on the ebb and flow of plasma which



TABLE 1  
*Shock produced by intravenous injection of adrenalin*

|                   | R. B. C. COUNT,<br>NORMAL | R. B. C. COUNT,<br>SHOCK | PER CENT INCREASE    | CORRESPONDING DE-<br>CREASE IN BLOOD<br>VOLUME, PER CENT<br>OF NORMAL | CORRESPONDING DE-<br>CREASE IN PLASMA<br>VOLUME | NORMAL BLOOD VOL-<br>UME, PER CENT OF<br>BODY WEIGHT | SHOCK BLOOD VOL-<br>UME, PER CENT OF<br>BODY WEIGHT | PER CENT DECREASE | REMARKS  |
|-------------------|---------------------------|--------------------------|----------------------|---|---|--|---|-------------------|--|
| A. Without acacia |                           |                          |                      |   |   |  |   |                   |  |
| I                 | 6,752,000                 | 8,804,000                | 30.4<br>45.0<br>max. | 23.4  | 39.0  | 9.5*   | 7.68  | 19.2              | This experiment is a compar-<br>ison to the succeeding for<br>comparison. Injection into<br>the femoral vein   |
| I                 | 7,096,000                 | 7,956,000                | 12.0                 | 10.7  | 17.8  |  |   |                   | In this and the succeeding 5<br>experiments adrenalin was<br>injected into a mesenteric<br>vein  |
| II                | 4,520,000                 | 4,630,000                | 2.4                  | 2.35  | 3.9   |  |   |                   |  |
| III               | 5,808,000                 | 5,800,000                | 0                    | 0   | 0   | 9.56   | 4.5   | 53.0              |  |
| B. With acacia    |                           |                          |                      |   |   |  |   |                   | Heart stopped 30 minutes af-<br>ter injection for shock vol-<br>ume determination. Cir-<br>culation continued 10 min-<br>utes by massage of the heart.<br>Pressure 41 with regular heart.<br>Typical shock tracing |
|                   |                           |                          |                      |   |   |  |   |                   | Adrenalin injection 5½ hours<br>after first count; 60 cc. blood<br>removed in samples. Shock<br>typical  |
| IV                | 5,856,000                 | 6,216,000                | 6.16                 | 5.82  | 9.7   | 10.7   | 7.25  | 32.2              |  |
| V                 | 6,656,000                 | 7,712,000                | 15.7                 | 13.5  | 22.5  | 9.8  | 6.56  | 33.0              |  |

| B. With acacia | VI<br>VII | 5,552,000 | 5,976,000 | 7.7 | 7.15 | 11.9 | 9.9 | 9.2 | 7.0 | Irregular heart<br>Shock determinations 23 min-<br>utes after fourth injection<br>of adrenalin. Pressure 22<br>mm. Hg. |
|----------------|-----------|-----------|-----------|-----|------|------|-----|-----|-----|--|
|                |           | 3,496,000 | 3,624,000 | 3.6 | 3.5  | 5.8  |     |     |     |  |

\* In this and subsequent tables the decrease in blood volume is figured as the reciprocal of the percentage of normal attained by the red blood cell count. The plasma decrease is estimated on the assumption that the plasma constitutes 60 per cent of the volume of the blood. In the last three columns are given the direct determinations of the blood volume by the acacia method. Where the normal volume is not determined it is assumed to be 9.7 per cent of the body weight, which is the average of the normal volumes of a long series of dogs.

result from the pressure changes, the concentrations being in this case 4, 8 and 11 per cent of the normal for the successive injections.

In experiment III of the acacia series a decrease in volume of 53 per cent by transudation alone would mean the loss of practically all the plasma. Obviously some other factors are involved in so large a depletion of the blood volume and of these, absolute stasis in some part of the vascular bed must be the most important. While the red cell count does not indicate any loss of plasma at all, this absence may be only apparent as the red cell count determines the minimum of concentration and the determination is misleading in proportion to the number of corpuscles that are jammed in the capillaries. In experiment IV, which was very satisfactory, a large difference between the absolute loss and the loss by concentration again occurs. That the difference is not merely apparent but may be due mainly to stasis, may be inferred after comparison with experiment VII in which, with a very small decrease in blood volume, such a degree of shock developed that the pressure was 22 mm. of mercury. Here undoubtedly the defective circulation must be attributed to stasis, which however falls short of being absolute.

In all but two of the experiments included in the table the adrenalin was injected into one of the small mesenteric veins. For this purpose care was taken to expose only one small loop of intestine. In these experiments curious localized constrictions of all branches of the mesenteric veins appeared which were permanent in position and gave the vein a sausage-like appearance. This phenomenon is probably one of the factors accounting for the loss of blood to the circulation by stasis.

#### SHOCK FROM CLAMPING THE AORTA

It was evident from the experience with adrenalin that in the other experimental procedures for producing shock the volume changes should be determined both without and with a previous injection of acacia. When no acacia had been injected clamping the aorta for three hours (table 2) produced a quite constant amount of concentration. As indicated by the red blood cell count the loss of plasma amounted to an average of 37.0 per cent of its original volume. On the contrary when acacia had been previously injected the plasma losses were 0 per cent and 8.8 per cent in two cases. Exactly similar results were obtained in the experiments in which the hemoglobin content was used as an index of the concentration (table 2 B., *expers.*

### Shock produced by clamping the aorta

|                   | R. B. C. COUNT,<br>NORMAL | R. B. C. COUNT,<br>SHOCK | PER CENT INCREASE | CORRESPONDING DE-<br>CREASE IN BLOOD<br>VOLUME PER CENT | CORRESPONDING DE-<br>CREASE IN PLASMA<br>VOLUME<br>OF NORMAL | NORMAL BLOOD VOL-<br>UME PER CENT OF<br>BODY WEIGHT | SHOCK BLOOD VOL-<br>UME PER CENT OF<br>BODY WEIGHT | PER CENT DECREASE | DE-<br>CALCULATED BLOOD<br>VOLUME | REMARKS  |
|-------------------|---------------------------|--------------------------|-------------------|---|--|---|--|-------------------|-----------------------------------|--|
| A. Without acacia | I                         | 6,150,000                | 30.8              | 23.7  | 39.5   |   |  |                   |                                   | Shown signs of im-<br>provement at end.  |
|                   | II                        | 7,624,000                | 24.1              | 19.4  | 32.3   |   | 8.4  |                   | 13.4                              | Not in shock<br>Blood volume not ob-<br>tained on account of<br>cardiac death. Volu-<br>minous mucohem-<br>orrhage exudate in<br>intestine       |
|                   | III                       | 7,424,000                | 31.0              | 23.7  | 39.5   |   |  |                   |                                   |  |
|                   | IV                        | 7,608,000                | 28.3              | 22.0  | 36.7   |   | 7.9  |                   | 18.5                              | Hemorrhage into intes-<br>tinal mucosa. The<br>count was taken be-<br>fore the clamp was re-<br>moved from aorta<br>From Hb. determina-<br>tions |
|                   | V                         |                          | 17.1              | 14.6  | 24.4   |   |  |                   |                                   |  |
| B. With acacia    | I                         | 6,240,000                | 0                 | 0   | 0  | 9.54  | 5.65   | 40.8              |                                   | Dog in profound shock<br>when clamp removed  |
|                   | II                        | 7,568,000                | 5.6               | 5.3   | 8.8  |   | 6.7  |                   | 31.0                              | No blood drawn before<br>acacia was injected   |
|                   | III                       |                          |                   |   |  | 10.0  | 7.56   | 24.0              |                                   |  |
|                   | IV                        |                          | 3.0               | 2.9   | 4.8  |   |  |                   |                                   | From Hb. determina-<br>tions   |
|                   | V                         |                          | 2.7               | 2.63  | 4.4  |   |  |                   |                                   | From Hb. determina-<br>tions   |

IV and V). Here again, as in shock produced by injection of adrenalin, the large differences between the actual decrease in volume and the apparent decrease due to loss of plasma, amounting respectively in two experiments to 40.8 per cent and 20.7 per cent, point to absolute stasis as the principal factor in decreasing the effective volume in these animals which had received acacia. In figure 2 are plotted the red cell counts obtained in two experiments performed for the purpose of comparing transudation under as similar conditions as possible except for the preliminary acacia injection in the one case. The concentration in the case without acacia was more than four times that in the control.

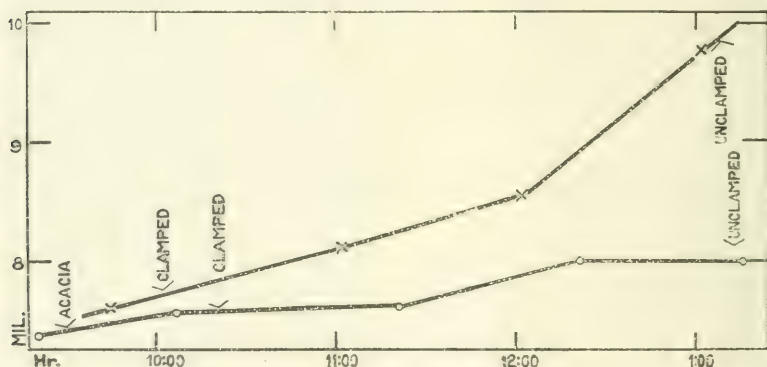


Fig. 2. R. B. C. count in shock produced by partial occlusion of the aorta. In the experiment indicated by the lower line 4 cc. per kilo of 20 per cent acacia were injected at the point designated.

#### SHOCK FROM MANIPULATION OF THE INTESTINES

In both of the two control cases (table 3), exposure of the intestines produced large concentrations of the blood. In these experiments the transudation of plasma could be observed directly on the peritoneal surface of the intestines. Beads of plasma would appear, grow larger, coalesce and start to run from the surface. In one experiment in which the drippings were collected, about 150 cc. were obtained. At the period of maximum transudation fluid was lost at the rate of 1 cc. per minute. This same phenomenon to a less degree can be seen also where shock is produced by other methods. If the abdomen is carefully opened droplets of serum are often seen on the peritoneal surfaces, especially of the liver and spleen.



TABLE 3  
*Shock produced by exposure of the intestines*

|                   |     | R. B. C. COUNT,<br>NORMAL | R. B. C. COUNT,<br>SHOCK | PER CENT INCREASE | CORRESPONDING DE-<br>CREASE IN BLOOD<br>VOLUME, PER CENT<br>OF NORMAL | CORRESPONDING DE-<br>CREASE IN PLASMA<br>VOLUME | SHOCK BLOOD VOL-<br>UME, PER CENT OF<br>BODY WEIGHT | CALCULATED DE-<br>CREASE IN BLOOD<br>VOLUME | REMARKS   |
|-------------------|-----|---------------------------|--------------------------|-------------------|---|---|---|---|---|
| A. Without acacia | I   | 6,664,000                 | 8,944,000                | 34.2              | 25.4  | 42.6  | 5.8   | 40.0  | Probable stasis in intestinal vessels<br>Loss mainly by concentration |
|                   | II  | 5,192,000                 | 6,904,000                | 33.0              | 24.7  | 41.1  | 7.5   | 22.7  |   |
| B. With acacia    | I   | 8,168,000                 | 8,504,000                | 4.1               | 3.95  | 6.6   |   | 32.0*                                       |   |
|                   | II  | 5,200,000                 | 5,920,000                | 13.8              | 12.1  | 20.2  |   | 10.0†                                       |   |
|                   | III | 5,536,000                 | 7,488,000                | 35.0              | 20.0  | 43.4  |   |   |   |

\* Approximation.

† Calculated on volume obtained after acacia injected without previous drawing of samples.

In this form of shock the difference between the experiments with and without acacia is not so marked. This may be due in the greater part to the fact that the damage produced can not be well controlled. The rapidity of the onset of shock in strong healthy animals varies with the severity of the manipulation. In one dog in the acacia series the concentration was maximal, in the other two the concentration amounted to less than one-half of that occurring in the controls. If we assume that the intestinal conditions are about the same in animals in which approximately the same time elapses between the exposure

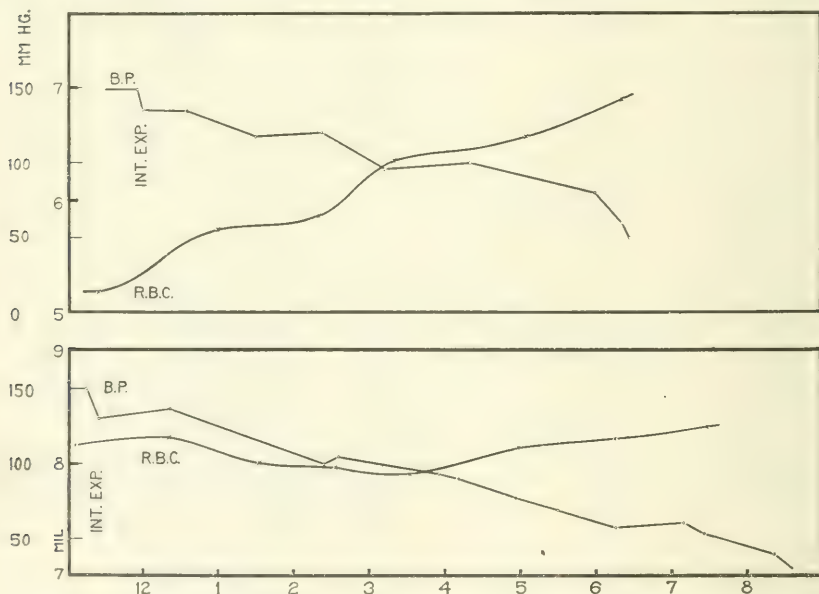


Fig. 3. Blood pressure and R. B. C. count in shock from manipulation of the intestines. Upper: without acacia. Lower: after the injection of 4 cc. per kilo of 20 per cent acacia.

of the intestines and the intervention of shock, then comparison of two such cases (fig. 3) shows that there is a considerably longer interval before the plasma loss becomes appreciable if acacia has been injected previous to the intestinal manipulation.

#### SHOCK FROM CLAMPING THE VENA CAVA

Four cases are included in this series (table 4). In all a concentration of the blood took place, the degree being somewhat greater in the two animals unprotected by acacia.

TABLE 4  
*Shock produced by clamping the vena cava*

|                        | R. B. C. COUNT,<br>NORMAL | R. B. C. COUNT,<br>SHOCK | PER CENT INCREASE | (CORRESPONDING DE-<br>CREASE IN BLOOD<br>VOLUME, PER CENT<br>OF NORMAL) | CORRESPONDING DE-<br>CREASE IN PLASMA<br>VOLUME | NORMAL BLOOD VOL-<br>UME, PER CENT OF<br>BODY WEIGHT | SHOCK BLOOD VOL-<br>UME, PER CENT OF<br>BODY WEIGHT | PER CENT DECREASE | CALCULATED DE-<br>CREASE IN BLOOD<br>VOLUME | REMARKS   |
|------------------------|---------------------------|--------------------------|-------------------|---|---|--|---|-------------------|---|---|
| A. Without aca-<br>cia | I                         | 6,816,000                | 24.0              | 19.8  | 33.0  |  | 8.4   |                   | 13.4  | Pressure 70 mm. Hg.,<br>animal improving.<br>Volume measured $\frac{1}{2}$<br>hour after last count<br>( ) = Maximum. Or-<br>gans especially hem-<br>orrhagic (Autopsy).<br>Volume determina-<br>tion corresponds to<br>smaller concentration |
|                        | II                        | 7,908,000                | 6.8<br>(21.3)     | 6.3<br>(17.6)   | 10.5<br>(29.3)                                  |  | 7.4   |                   | 23.7  |   |
| B. With acacia         | I                         | 6,456,000                | 2.3               | 2.3   | 3.8   | 9.8  | 9.0   | 9.2               |   |   |
|                        | II                        | 6,400,000                | 12.8              | 11.3  | 18.8  |  | 8.5   |                   | 16.0†                                       |   |

† Calculated on volume after acacia injection.

In the second experiment in series A, the concentration of the blood was progressive until the clamp was removed. After this the red cell count decreased. At the end of the experiment the blood volume as determined directly was 23.7 per cent less than normal. If the circulation had been recovering as the cell count might indicate the pressure would not have progressively fallen and the final volume would not have been so low. A more probable explanation is that some of the corpuscles were jammed in the capillaries and therefore caused a decrease in the count, a view which is supported by the hemorrhagic condition of the organs at autopsy. In another series of nine animals in which the cava was clamped for 2 to 2½ hours so that the general blood pressure was 40 mm. of mercury, the concentrations at the end of clamping as indicated by hemoglobin determinations averaged 117.6 per cent of the normal, the individual variations ranging from 110.4 to 131.8 per cent.

#### DISCUSSION

In every case studied shock was associated with a concentration of the blood and loss of volume. This was not a phenomenon of severe shock alone, but started soon after the procedure was begun, the purpose of which was the production of shock. The concentrations did not always vary parallel to the loss in absolute volume.

In the slightly larger portion of the total number of experiments the total loss of volume can in the main be explained by the amount of plasma loss. Examination of the tables shows that in a few cases the shock volumes as determined directly are not quite small enough to coincide with the volume calculated from the red cell count. These differences are without significance as they are obviously errors in procedure and technique and the reasons for their occurrence must vary with the individual cases. The following possibilities may be mentioned: 1, In one case the direct determination was made one-half hour after the red cell count, in an animal that was recovering. 2, When the volume of the blood in shock is determined without a previous determination of the normal volume, the percentage decrease is calculated on the assumption that the normal volume was 9.7 per cent of the body weight, although in some cases it might have been actually higher than the average for normal dogs. 3, It is probable that if we had followed the hemoglobin content instead of the cell count, many of the differences would have disappeared as it has been our experience that the variations in volume calculated from the

erythrocyte count were almost uniformly greater than those calculated from simultaneous hemoglobin determinations. Scott, Herrmann and Snell (5) had a similar experience in their studies of the concentration of the blood after muscular exercise. These authors give a discussion of this point with a citation of the literature.

In the other group, cases can be found in which the total loss of blood as determined by the acacia method is much greater than that indicated by the red cell count. The greatest variations between the total loss as actually determined and that deduced from the concentration were seen in the adrenalin cases in which the injections were made into a mesenteric vein. In this condition the venous constrictions mentioned above appear as a special factor. In three cases where the total losses were 53, 32 and 33 per cent of the total blood respectively, the losses by plasma filtration were 0, 6 and 13 per cent. There can be no doubt that this special condition can only be incidental and does not even account for the differences found in this particular type of shock. As has been already mentioned, in two cases after clamping the aorta there were differences of 40 per cent and 20 per cent of the blood volume between the total loss and the loss by transudation. Similar cases also occur in shock from clamping the cava or manipulating the intestine, although the differences seen are not so great.

Before discussing the manner in which this blood is lost, it is necessary to review the post-mortem findings. In over one hundred animals examined it was found that no matter how the shock was produced the same general picture obtained. The most constant finding is an injection of the intestinal mucosa. In the milder cases the injection is confined to the duodenum; in more severe cases it extends along the whole of the small intestine giving it the appearance of deep red velvet. In the lower ileum the injection often again disappears although in severe cases it extends up to the ileo-cecal valve. In 60 per cent of the cases blood was found in the intestinal lumen; this was at times due to isolated punctate hemorrhages, more often a general sloughing of the tips of the villi took place. Microscopic sections show that except at injured tips of the villi the corpuscles are almost entirely within the vessels. The capillaries and small veins are greatly dilated and tightly packed with red blood cells. It is interesting to note that in animals that recover from this condition the injection has disappeared. How much this is due to sloughing and how much to clearing out of the capillaries we are unable to say. The spleen is usually swollen and has dark raised areas which consist of hemorrhages into the pulp. This



swelling and hemorrhage in some cases produces a spleen many times the normal size. The liver is occasionally found to be hemorrhagic on section.

Where the loss of blood volume as directly determined is much greater than that determined by the red cell count, the difference can be attributed mainly to stasis. The red cell count is an index only to loss of plasma, as a result of which concentration of corpuscles takes place. As long as no other factor enters, the blood volume can be properly interpreted in this way. But as soon as the corpuscles reach a certain concentration, 60 per cent according to Trevan (6), they become contiguous, the internal friction rises rapidly and they have a tendency to jam especially when the arterial pressure is decreased as it is in these experiments. Insofar as the corpuscles are blocking the capillaries they are not included in the determinations of the red cells or of the hemoglobin content, and the latter fail as indices of blood out of circulation by the volume of the corpuscles and the volume of the plasma that would normally accompany them. In addition to corpuscular sedimentation the difference may be attributable to sequestration of both corpuscles and plasma and to blood lost into the intestinal lumen and to a much less extent to hemorrhages into the spleen pulp.

In the group in which the total blood loss is to be accounted for by transudation, the congestion of the capillaries and the small veins is again seen post mortem; in these dilated and congested areas the blood must therefore be moving, however slowly.

The blood volumes found in severe shock are often remarkably high. Typical shock was found to occur in animals whose absolute volumes were decreased by only 7 to 17 per cent of the normal. For this there can be only one interpretation and that is an enlargement of the vascular bed and, therefore, a greatly reduced effective volume. Mann (7) in his experiments found that in shock from exposure of the intestines a portion of the blood becomes immobilized. On the assumption that the blood volume is 7.7 per cent of the body weight, he found that when all the blood that could be obtained was drawn from the femoral artery and heart, 24 per cent of the blood remained in the tissues of the normal dog while under similar conditions 61 per cent of the blood remained in the tissues of the dog in shock. We have often observed a similar phenomenon. From a dog in shock weighing 16 kilos, which would normally contain about 1400 cc. of blood, only 100 cc. could be obtained although artificial respiration and massage of the heart were resorted to. The decreased amount obtainable by bleeding is

in part due to the decreased volume, but analysis of Mann's data shows clearly that after allowance for the decreased volume of the blood in shock the actual amount of blood left in the body is more than in the normal dog. These findings are in line with the observations that have been reported by surgeons that fatal consequences may supervene on the losses of apparently trivial amounts of blood. The experimental animals maintain their pressure in the face of decreasing blood volume to what may be called a breaking point beyond which the pressure rapidly fails and death soon results.

In spite of the fact that the amount of blood which may be obtained by bleeding is so small (i.e., that so little of it can be moved from the stagnant area by the remaining *vis a tergo* after hemorrhage is started), it is still possible for the shocked animal to gradually move the mass of blood in the stagnant area so that an injected substance, as acacia, is gradually mixed with the total circulating blood. The figures for the blood volumes represent all the blood that passes through the heart in a period of ten minutes, and as indicated in the preceding paragraph, may amount to a total not far below normal. While the evidence indicates that the deficiency of the circulation is mainly due to sluggishness of the flow in some parts of the vascular bed, the work of Gesell (8) shows that a decrease of 7 to 17 per cent of the blood volume is itself of significance. He found that a loss of blood by hemorrhage of less than 10 per cent may elicit through constriction of central origin a decrease of flow through the submaxillary gland of more than 60 per cent.

After simple hemorrhage the volume would rapidly be made up from the tissues and the flow restored. In these experiments, on the other hand, the volume-compensating mechanism is becoming exhausted and the decrease in volume, though small, is sustained. If the constriction would be sustained, then the reduction in flow might cause damage to the parts of the body compensating the areas primarily injured. Robertson and Bock (9) have found that after a large hemorrhage in wounded soldiers the blood volume even when aided by transfusion may not return to normal for  $2\frac{1}{2}$  days. The organism is, therefore, able to maintain its existence after large losses of blood without complete restoration of the volume for several days.

The locus in which the plasma is stagnant is certainly not the arterioles or portions of the vascular bed proximal to them. In the direct determinations of peripheral resistance to the inflow of salt solution (10) under a constant high pressure, it was found that while the periph-

eral tone was decreased when the pressure falls below 50 mm. Hg, in the periods preceding this low level the resistance may be but very little decreased, normal or even above normal. The condition of the large veins in the abdominal cavity can be easily noted by direct observation. Workers with experimental shock are agreed that in this condition the veins are collapsed and not engorged. There is left, then, only the capillaries and small veins. The full dilated capillaries and venules seen in the microscopic sections from the organs in shock prove their involvement beyond peradventure. It is interesting to note that Mall and Welch found that in the dog's mesentery after occlusion of the superior mesenteric artery the smaller veins become distended with corpuscles even before the capillaries. An opening up of normally unused capillaries such as occurs in inflammation may also well be a factor in enlargement of the capillary bed, as has been suggested by Cannon (11).

If one reviews the whole series of shock experiments and compares the concentrations obtained where shock was induced with and without an early injection of acacia, he finds that the average reduction of the blood volume by plasma loss was 6.8 per cent in the former case as compared with 20.3 per cent in the latter. It might be expected from such data that the animals that receive acacia would be more difficult to put into shock than their controls. There was, however, nothing observed in this series to contribute to this view. While on theoretical grounds it is to be expected that any benign method of interfering with transudation would be invaluable in the maintenance of normal blood volume, it should be fully appreciated that transudation is only one of the attendant circumstances in the development of the shock picture as a whole. That the transudation may be greater than indicated by the cell counts and may, therefore, alter the quantitative difference between the two series somewhat, should not be overweighted as the same phenomenon occurs in both series.

To explain the conserving action of the acacia on the plasma volume a consideration is necessary of the factors that might determine its loss. The loss of plasma might be due to a decreased colloidal content, to an increase of capillary pressure or to a change in permeability in the vascular endothelium.

*The protein content of the plasma* was studied in shock from clamping the aorta by determining its specific gravity with the pycnometer and its power of refraction with the Pulfrich refractometer. The latter method is theoretically more accurate as the salts have a relatively

greater specific gravity than protein but a smaller refractive index. The findings obtained with one instrument in the main confirm those obtained with the other. They show the same sequence of events in each case (table 5). There is in two experiments a slight increase of the protein content up to the time that the clamp is removed; in the other two experiments the increase is absent. Water evidently filters more rapidly than protein at first but after unclamping when the pressure is raised in the splanchnic region this is no longer true, the increased

TABLE 5

| EXPERIMENT | BLOOD PRESSURES | HEMOGLOBIN PER CENT OF NORMAL | SPECIFIC GRAVITY OF SERUM | PER CENT OF PROTEIN IN SERUM (REFRACTOMETER) | REMARKS                |
|------------|-----------------|-------------------------------|---------------------------|--|------------------------|
| I          | 105             | 100.0                         | 1.0256                    | 5.902  | Normal                 |
|            | 58              | 112.4                         | 1.0261                    | 5.967  | After 3 hours clamping |
|            | 40              | 113.3                         | 1.0252                    | 5.553  | Final                  |
| II         | 105             | 100.0                         | 1.0275                    | 7.049  | Normal                 |
|            | 58              | 111.4                         | 1.0277                    | 7.049  | After 3 hours clamping |
|            | 40              | 111.4                         | 1.0272                    | 6.768  | Final                  |
| III        | 180             | 100.0                         | 1.0284                    | 6.970  | Normal                 |
|            | 100             | 114.1                         |                           | 7.416  | After 3 hours clamping |
|            | 43              | 121.6                         |                           | 6.719  | After second clamping  |
|            | 43              | 122.5                         | 1.0274                    | 6.465  | Final                  |
| IV*        | 100             | 100.0                         |                           | 7.675  | Normal                 |
|            | 77              | 102.1                         |                           | 7.675  | After 3 hours clamping |
|            | 30              | 101.6                         |                           | 7.653  | Final                  |

\* This animal received 4 cc. per kilo of 20 per cent sodium acacia before the aorta was clamped.

permeability allows filtration of more protein and this increase is followed by a change in the opposite direction so that the protein content is finally 4 to 7 per cent less than at the start. It therefore follows that the plasma is lost mainly as a whole (see also Dale and Laidlaw (12) ), and that a change in the colloid content of the plasma is not an important factor in the filtration as the latter process occurs during the period in which the colloid content is increasing as well as in the later period when the plasma is again diluting.

The explanation of the dilution in the final period probably lies in the fact that the organism is attempting to compensate the decreased



volume. The decrease in the protein content of the plasma would accord with the assumption that the passage of fluid from tissue to blood is by osmosis and therefore consists mainly of water and salts (13). As the loss of plasma is progressive all parts of the vascular bed are not similarly affected; in some portions the normal reaction to decreased volume is possible synchronously with a condition allowing continuous filtration in other portions. The compensation of the loss of fluid by normal tissues was first noted by Cobbett and Roy (14). Our data give the additional information that some areas are still compensating even when the blood is concentrating, though with insufficient rapidity to keep pace with the filtration.

The filtration of the plasma as a whole precludes the possibility that one finds suggested in the shock literature that the plasma is depleted by an increased affinity of the tissues for water.

In many cases a rise of capillary pressure must be a factor. After adrenalin the observed (15) rise of portal pressure must determine a rise in the mean capillary pressure of the intestinal area. When the cava is clamped there is a slight rise of portal pressure while the venous pressure in the liver which is normally very low must be relatively greatly increased. When the aorta is clamped it is difficult to see where in the splanchnic area the capillary pressure can be increased unless it be in capillaries plugged at their distal ends. Aside from this possibility transudation in this condition must be due to a change in vascular permeability. This latter condition is seen most clearly when the intestines are manipulated. Here, to be sure, alterations in pressure may also occur from various mechanical possibilities but the important changes are those which also occur in the inflammatory process, the analogy to which has been noted by a number of observers.

Regardless of the cause of shock, it would be expected that the expansion of the blood volume, which would result from the effort of the organism to compensate the increase of colloidal osmotic pressure of the blood produced by the injected acacia, would aid in maintaining the volume. To test the degree to which this might take place, data on the osmotic pressures developed by acacia and blood serum are necessary.

*Osmotic pressures of acacia and blood serum.* As Bayliss has correctly pointed out the determinations should be made against Ringer's solution. The two variables, acidity and  $\text{CO}_2$  content of the solution, must be controlled. A difficulty also arises in the fact that acacia is altered by the presence of dilute sodium bicarbonate solutions. Natu-



ral acacia is combined mainly with calcium. Of the other constituents magnesium, another divalent cation, predominates. When acacia is added to a dilute sodium bicarbonate solution, some of the divalent cations are replaced by sodium, as is shown by the development of a precipitate. This takes place slowly if made up in the cold, more rapidly if warmed. We have found that sodium acacia develops twice the osmotic pressure that natural acacia does. This is true whether determined directly against water or by cryoscopy of concentrated aqueous solutions.

The osmotic pressure will depend on the nature of the cations with which it is combined when it comes to equilibrium in the blood. While we have not determined the quantitative effect of all the variables, we believe that we obtained a fair approximation of the natural conditions by dialyzing the acacia against Locke's solution without glucose but containing enough sodium bicarbonate so that when in equilibrium with a partial pressure of 40 mm. of  $\text{CO}_2$  the reaction was about  $\text{P}_{\text{H}}$  7.4. The acacia was made up to a 7 per cent strength in the above solution at room temperature. It was dialyzed in a Moore and Roaf osmometer (celloidin membrane) against the buffer mixture, arrangement being made for a continuous change of fluid in the lower chamber. Under these conditions the osmotic pressure of the 7 per cent solution was found to be 22 mm. of mercury.

Sodium acacia similarly prepared developed a pressure of 28.7 mm. of mercury.

These figures are evidently lower than those obtained by Bayliss (16). The difference is probably to be attributed to the differences in the other substances existing in the solution. A wide range of pressures may be obtained according to the conditions of solution; for example, 6 per cent sodium acacia developed a pressure of 274 mm. of mercury in two days against  $\text{CO}_2$ -free distilled water; the water in the lower chamber was then changed to water saturated with  $\text{CO}_2$  at atmospheric pressure and the osmotic pressure of the acacia fell to 83 mm. by the end of two weeks.

The osmotic pressures obtained with fresh dog serum dialyzed against the buffer mixture were also found to be lower than those usually reported, being 16.4 mm. of mercury as compared with 25 to 30 mm. for serum dialyzed against its ultrafiltrate (13), 27 mm. for pig serum against 0.9 per cent saline (17), and 36 to 40 for ox serum against Ringer's solution (16).

*Theoretical considerations as to the effect of the injected acacia on the osmotic properties of the plasma.* On the basis that the osmotic pressure of 7 per cent acacia is 22 mm. of mercury and of the protein of the blood serum 16.4 mm., 4 cc. of 20 per cent acacia would have to expand to  $\frac{(20 \times 22 \times 4)}{7 \times 16.4}$  or 15.3 cc. to become isotonic to the serum colloids. This would mean a 16.6 per cent expansion of the blood volume (normal = 92 cc. per kilo). When 4 cc. of 20 per cent acacia were added to 54 cc. of serum and dialyzed against the same serum, the mixture developed a pressure of 3 mm. of mercury, being, therefore, 18.3 per cent hypertonic, and in experimental agreement with the calculation made from the osmotic pressures of serum and acacia determined separately.

The normal volumes and red cell counts were determined ten minutes after the acacia was injected. At this time the expansion of the blood is never more than 2 to 3 per cent above the calculated simple dilution by the volume injected. If we subtract this 6.4 per cent (4.4 per cent expansion by the volume injected + 2 per cent expansion due to dilution) from the 16.6 per cent increase in volume theoretically possible after the injection, there remains a further possible expansion of 10.2 per cent of the blood volume. The water which theoretically might be attracted by the acacia might be expected to account in part for the differences in plasma volumes in shock induced with and without a previous injection of acacia, but there is no evidence that the filtration is masked by a progressive dilution of the injected acacia. In the normal dog after the injection of 4 cc. per kilo of 20 per cent acacia, the expansion of the blood is always less after one hour than it is after ten minutes, not more. The maximum actual expansion never reaches anything like the theoretical. This point is of considerable interest as it minimizes the importance of what would seem to be one of the most obvious explanations of the protective action of acacia, namely, its increase in volume by the attraction of water.

When the procedure whose purpose is the induction of shock is not started until one hour after the acacia injection, the conserving action of the acacia on the plasma volume is just as apparent. At the end of one hour as the blood has not expanded by absorption from the tissues and as direct chemical determinations have shown that at this time the blood contains about 92 per cent of the amount present at the start, the blood colloids must have an osmotic pressure greater

than normal (calculated<sup>1</sup> to be 17 per cent greater). This fact gives a clue to its probable action.

When the capillary filtration pressure is high it is opposed by the increased colloidal osmotic pressure as is strikingly seen in the adrenalin experiments. In conditions of increased permeability, other conditions being equal, the increase of filtration would again be opposed by the increased pressure of the colloids. This would be the case in the experiments where shock was produced by clamping the aorta. In cases where the injury to the vessels is sufficient acacia is apparently without beneficial effect. This is seen in experiment III in the shock series from manipulation of the intestines. We have also had a similar experience in some experiments with pulmonary edema resulting from the inhalation of irritating substances.

While the expansion of the blood is slight compared with what is theoretically possible, the blood plasma is potentially more able to take fluid from the tissues should such a demand arise. Evidence has been presented that the decreasing volume of the blood resulting from trauma is partially compensated by fluid from uninjured tissue. This process would be aided by the osmotic pressure of the acacia in the plasma and much more fluid would have to be absorbed from the tissues before the concentration of the blood colloids would go below normal.

Acacia contains calcium in ionizable form as it may be precipitated by oxalates. That calcium is combined with a large organic element and is not present as a salt impurity is indicated by the fact that the acacia can not be freed of the calcium by dialysis. The possibility that the inhibitory action on permeability might be due to calcium has been considered. If the calcium were the sole factor concerned the action would not be shared by sodium acacia. The latter, however, is not the case as sodium acacia has an undoubted protective action; in fact our most recent observations show that when either sodium or calcium

<sup>1</sup> Calculation.

$55/59 \times 16.4 = 15.28$  (osmotic pressure of plasma when diluted with 4 cc. of water per kilo; plasma volume assumed to be 60 per cent of 92 cc. of blood per kilo).

4 cc. of 20 per cent acacia yields a 1.356 per cent solution in 59 cc. of water.

$1.356/7 \times 22$  (osmotic pressure of a 7 per cent sol.) = 4.26.

$4.26 \times 0.92 = 3.92$  (osmotic pressure of the acacia in the plasma 1 hour after injection).

$15.28 + 3.92 = 19.2$  (total osmotic pressure of plasma 1 hour after injection).

$19.2/16.4 = 117$ , therefore the osmotic pressure of the plasma would be 17 per cent greater than normal.



acacia is dialyzed against Locke's solution containing sodium bicarbonate in equilibrium with alveolar air and with a reaction of  $P_H$  7.4 that their osmotic pressures approach each other and presumably, therefore, when either form of acacia comes to chemical equilibrium with serum, the product is the same. Calcium action could arise, therefore, only from the salt resulting from the liberated ion.

#### SUMMARY

The blood volume was determined in shock by the method of Meek and Gasser and compared with the reduction in blood volume as determined by the enumeration of the red blood corpuscles.

The blood volume was found to be decreased in all forms of experimental shock studied and after all grades of damage.

Red cell counts or hemoglobin determinations are of value in indicating blood volume only when no absolute stasis occurs and when no corpuscles are jammed in the capillaries.

The effective volume of the blood may be reduced in the following ways:

1. By decrease in the volume of the blood as the result of:

- a*, Transudation of plasma.

- b*, Transudation of plasma and jamming of the corpuscles in the capillaries and venules, or the latter combined with

- c*, Absolute stasis in some part of the vascular system.

- d*, Hemorrhage into tissue, especially into the lumen of the intestines.

2. By dilatation of the capillaries and small veins with greatly decreased slowing of the circulation. This is always attended by some loss of plasma, but the latter may be relatively inconsiderable.

The transudation of plasma is greatly opposed by injection of 4 cc. per kilo of 20 per cent acacia before traumatization. The mechanism of action is believed to be due mainly to the antagonism to filtration by the resulting increase in the osmotic pressure of the plasma colloids. A discussion is given of the other possibilities.

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## THE CATALASES OF THE BLOOD DURING ANESTHESIA

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There are two important theories in explanation of the mechanism by which anesthetics produce anesthesia. The one states that they produce their effects by diminishing the permeability of cell membranes (1). The other theory, that of Verworn and his followers (2), states that anesthesia is produced by an inability of cells to use oxygen. This inability is ascribed to depression of oxygen "activators" or oxygen enzymes. Our interest centered about these theories, and was renewed by publications in which it was said that the catalases of the blood were diminished in anesthesia. Added to this was the statement that this discovery forged another link in the chain of reasoning of the Verworn school (3).

Catalases may be defined as substances, if substances they are, which break up peroxides into molecular and thus inactive oxygen, and water. The function of catalases, according to most views, is to destroy peroxides when their presence would be harmful in the organism. Others subscribe to this effect, plus certain specific ones, such as the release of oxygen from hemoglobin. Von Furth however, who reviews the evidence, sums up by saying that "we not only know nothing positive about the physiological operation of the catalases, but also that we do not even know whether they have any important physiological significance at all, and whether they may not perhaps be altogether accidental and non-essential" (4). Burge (5), however, who with his associates has published numerous articles on this subject, claims great power and physiological significance for these catalases. The immediate ones which interest us are that they are decreased in anesthesia, according to his results, and that, since they are decreased, they are at least one of the causes for the decrease of oxidation in anesthesia, and hence for anesthesia itself. The interpretations and far-reaching conclusions which have been deduced from his results of catalase determinations under various conditions have seemed, *a priori*, too all-

conclusive. Recently F. C. Becht (6) has so ably and justly criticised not only his interpretations, but also his analytic data and methods, that it is unnecessary to repeat specific considerations here. Suffice it to say that we have met with the same difficulties in technique and in obtaining consistent results which Becht speaks of. The errors encountered in using these methods will be obvious to any one after a few trials. Our technique has not been as elaborate as that of Becht, but more so than that described by Burge. Blood was collected from an arm vein before anesthesia and immediately after operation was completed and the anesthetic stopped. Oxalate was used to prevent coagulation. The patients were unselected and were operated for the usual surgical diseases in the clinic of Dr. John B. Deaver. All determinations were made within a very short time after the collection of blood; 0.5 cc. of blood was added to 100 cc. of distilled water at 37°C. plus or minus 2°. The vessel containing this was immersed in a water bath at 40°, plus or minus 2°. Fifty centimeters of hydrogen peroxide were then added through a glass stoppered thistle tube passing into the bottle containing the blood and water. The evolved oxygen was collected through a second tube in an inverted burette. The apparatus containing the blood, water and hydrogen peroxide was shaken in a machine at a constant rate of one hundred and seventy-five double shakes per minute. The amount of oxygen evolved, first in 10 minutes, in later experiments in 5 minutes, was taken, reduced to 0°C. and 760 mm. pressure, as the measure of the catalases of the blood. The results in the table are typical of two hundred determinations, many done in duplicate, some in triplicate, and others done as many as four or five times. The same sample of hydrogen peroxide was used for the same individual before and after anesthesia, and as many constant conditions such as temperature, shaking, etc., were introduced as possible. Even so, to say nothing of the blood from different individuals, the blood from the same individual in duplicate or triplicate determinations gave results frequently differing by 50 per cent, and even 100 per cent, differences as large as in determinations before and after anesthesia. Of the whole number in which the most consistent results were obtained, 35 per cent showed an increase of catalases and 65 per cent showed a decrease. Granting that the analytic data are accurate, since the catalases were not universally diminished, they cannot, after all, play such an important rôle in anesthesia. Since, however, these methods of determining catalases are so highly inaccurate and varying, conclusions drawn from such observations can have no weight in physiological deductions.

TABLE I

*Catalases of the blood, before and after anesthesia. Cubic centimeters of oxygen evolved*

| BEFORE | AFTER | BEFORE | AFTER |
|--------|-------|--------|-------|
| 316    | 381   | 418    | 353   |
| 417    | 410   | 430    | 412   |
| 92     | 32    | 250    | 379   |
| 372    | 414   | 55     | 179   |
| 841    | 1116  | 370    | 357   |
| 35     | 13    | 935    | 890   |

## SUMMARY

Estimations of the catalases of the blood have been made before and after anesthesia. They were decreased in 65 per cent of the cases and increased in 35 per cent of cases.

The method for the determination of catalases has been criticised and the opinion expressed that they are inaccurate, and that no deductions can be drawn from them. Our results before and after anesthesia come within the experimental error (7).

The functions of catalases in the body are unknown.

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# CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

## LI. THE CONTROL OF THE PYLORUS

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### INTRODUCTION

From the earliest times anatomists and physiologists were so impressed with the "peculiar office and functions of the pylorus" that

Van Helmont conceived it to be the peculiar seat of the soul, an opinion to which Willis gave a degree of support. Richerand ascribed to it something like intelligence, when he said that it has a peculiar tact which enables it to select from the contents of the stomach what is proper to pass through, while it rejects the remainder (1).

The more rational explanations which followed were essentially mechanical in nature but not entirely satisfactory. More satisfactory was the theory proposed by Cannon (2) that the pylorus was under the chemical control of the acid chyme. The wealth of conclusive experimental evidence in support of this theory leads early to its acceptance by clinicians and physiologists, so that at the present day its general correctness is not doubted. But Cannon (3) himself stated that other factors might modify the chemical control, especially under abnormal conditions. To this many observers will readily agree and will quickly point out that Cannon's theory not only fails to explain the emptying of the stomach in achylia gastrica but that Cannon never convincingly explained the rapid exit of  $H_2O$  and neutral egg white solution which left the stomach before the free acidity of the intragastric contents was present to effect an opening of the pylorus or sufficiently concentrated after ejection into the duodenum to effect its closure. More recently, Morse (4) reports a diminution in the rapidity of discharge of the stomach with an increase in the acidity of the gastric contents—quite opposed to the findings of Hedblom and Cannon (5)—but explains the discrepancy on the basis of difference in experimental method.



Spencer, Meyer, Rehfuess and Hawk (6) observed that 1.0 per cent sodium bicarbonate hastened the discharge from the normal human stomach. On the motor side, Carlson (7) reports an inhibition of hunger contractions by introduction into the stomach of both acid and alkaline solutions but that the inhibition is greater with a given concentration of hydrochloric acid than with a corresponding solution of sodium bicarbonate. The experimental conditions of Cole on human beings quite closely approximate those of Cannon, made for the most part on the cat. Cole (8) finds that a meal of mixed foods and fluids begins to leave the stomach immediately after ingestion, "certainly before ingestion of a full meal is complete." Cole emphasizes here, as in a previous article (9), that the pylorus opens partly at each "systole" and that "during the stage of "diastole" when the gastric pressure is diminished, the sphincter may be closed to prevent the chyme from falling into the stomach." In short, he correlates the opening of the pylorus with the passage of peristaltic waves, and an increased tonicity of the stomach. Ortner (10) ascribes the opening of the pylorus to an optimum dilution of the gastric contents rather than to the presence of free acid in the antrum. Egan (11) corroborates Cole's findings that in health as well as disease the first portions of the food ingested by the fasting stomach may leave it at once.

The present short contribution likewise establishes an interdependence between the opening of the pyloric sphincter and the tonus rhythm of the stomach and emphasizes anew together with observations of other authors that certain motor activities of the stomach are intimately associated with the relaxation of that sphincter.

#### METHODS

The observations which led to this conclusion were obtained partly from fluoroscopic observations of the stomach of man (A. J. C.) following the ingestion of an emulsion of tragacanth carrying in suspension  $\text{BaSO}_4$ , while recording the motor activities of the stomach by the balloon method partly from observations on normal fasting dogs provided with a gastric and duodenal fistula. In the animals the duodenum was attached to the anterior abdominal wall about 1 to 2 cm. from the pylorus at the time of making an ordinary gastrostomy. Following the uneventful recovery from this double but simple operation, a small opening was made into the duodenum. Through the gastrostomy we introduced into the dog's stomach  $\text{H}_2\text{O}$ , milk, milk-



peptone solution, or a 2 per cent cooked starch suspension as such or after the addition of an amount of dog's gastric juice sufficient to show presence of free acid with a few drops of congo red solution. The balloon was next inserted through the gastric fistula and attached suitably to the manometer in the usual fashion (12). With the dog lying comfortably on its right side in the lap of an assistant we next introduced through the duodenostomy a piece of rubber tubing with opening directed toward the pylorus and with its tip no more than 1 cm. from that sphincter. Following a short period of temporary inhibition as a result of these preparatory manipulations, the dogs evinced a typical tonus rhythm. Each drop of fluid issuing from the tube in the upper part of the duodenum was registered immediately beneath the manometer tracing of the gastric activity by means of a signal magnet. Very often the drops issued in so rapid a succession that the record of the individual drops merged into a broad continuous band.

In man, the balloon was swallowed just prior to the ingestion of about 10 ounces of a drink of aqueous emulsion of tragacanth carrying in suspension  $\text{BaSO}_4$ . The subject was placed on the X-ray table, face down, and after attaching the rubber tubing from the balloon to the recording manometer, fluoroscopic observations were made on the passage of the test drink through the pylorus and into the duodenum. As soon as the duodenal cap filled and the dark mass quickly passed into the upper portion of the duodenum the fact was recorded by a signal magnet writing immediately below the writing point which was recording the gastric activities at the time and just prior to the opening of the pylorus.

#### RESULTS

The direct fluoroscopic observations in man showed plainly in confirmation of the contention of Cole (8) and others that the fluid contents were carried past the sphincter and into the duodenum just as soon as we could prepare the patient to record the fact. Peristaltic waves would pass over the stomach starting approximately in the middle of the body (13) of the stomach and course toward the pylorus. The pyloric sphincter would open and the dark mass would be hastily passed through the whole course of the duodenum. Figure 1 illustrates the record given during the period of such observations. It will be noted that the period of opening of the pylorus and the passage of fluid contents through the duodenum as recorded by the lowermost line is coincident with the record of tonus rhythm of the stomach which



hunger contractions which are admitted by Rogers and Hardt to result essentially from peristalses starting at the cardiac orifice and passing "toward the pyloric end as a rapid peristaltic wave."

In short, the tonus contractions of the stomach accompanied by visible peristalsis (the "systole" of Cole) (15) effected an opening of the pyloric sphincter with egress of the gastric contents into the duodenum. In the dogs we recorded the drops of gastric contents issuing from a tube placed in the duodenostomy by a signal magnet writing just below the flag of the manometer recording stomach contractions after partly filling the stomach with  $H_2O$ , acidified starch paste or milk peptone mixture.

As soon as we had prepared the animal for the registration of the results, the stomach began to empty itself of its contents which were

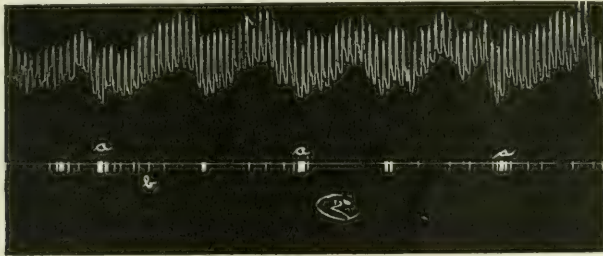


Fig. 2. Tracing showing the movements of the stomach containing food in a fluid condition. The signal magnet tracing below records the drops of fluid issuing from a duodenostomy located within an inch of the pylorus (dog).

often tinged with some bile. The fluid was acid towards phenolphthalein, rarely acid to congo red and dimethylamidoazobenzene. Though usually tinged with bile we could easily demonstrate the presence of starch and absence of sugar precluding the possibility of regurgitation of mixed intestinal contents (pancreatic juice, bile and succus entericus). It was, furthermore, never alkaline to phenolphthalein as noted above.

More interesting to us was the relation existing between the time of opening of the pyloric sphincter and the motor activity of the stomach. Figures 2, 3 and 4 are submitted to illustrate this relationship. Figure 2, obtained from a dog, is quite similar to figure 1 obtained from one of us (A. J. C.). In both instances the fluid is seen to issue during the rise in the intragastric pressure known to be due in the latter case to waves of constriction passing toward the pylorus (peristalses). As

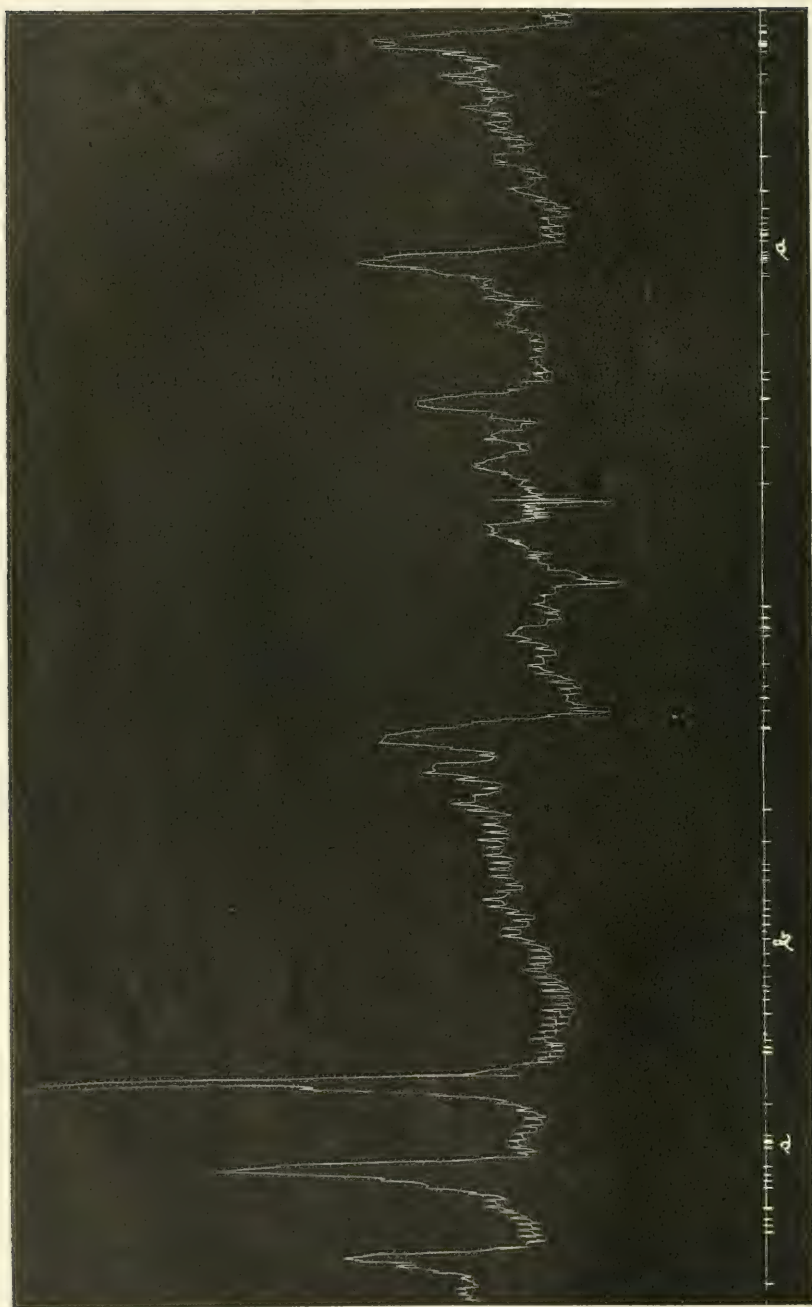


Fig. 3. Record of the movements of the partly-filled stomach with a signal magnet tracing below indicating the egress of the gastric contents through a duodenostomy located just below the pyloric sphincter.



the tracing plainly shows, the drops issued most abundantly at a time when the constricting ring had reached the pylorus. It may and does very often issue during the rise in tone of the stomach which may be due not entirely to a traveling ring of constriction (peristalsis) but to general increase in tonic activity of the musculature as a whole as is seen at *b* in figure 2, and better still at *b* in figure 3. Certain it is that the pylorus is most widely open, if judged by the quantity of intra-gastric contents issuing from the tube in the duodenum, at the

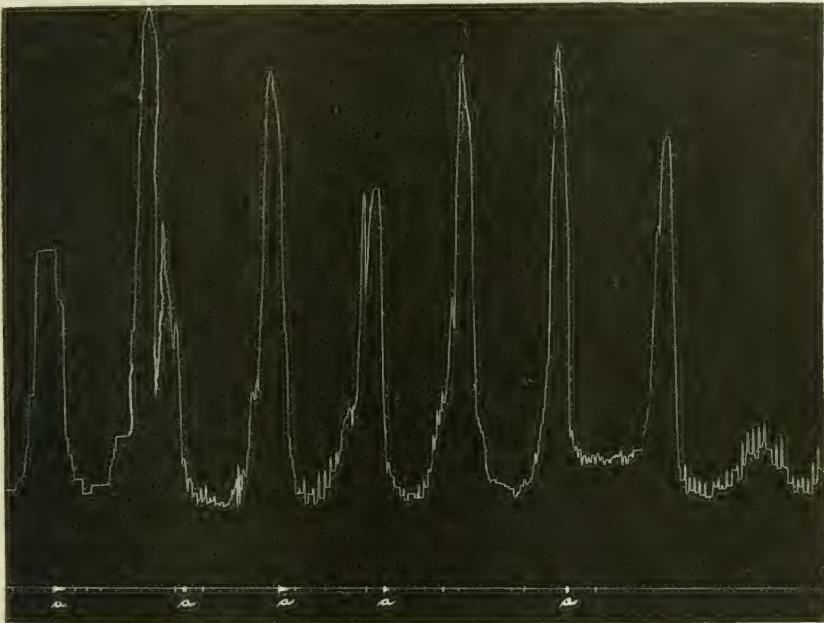


Fig. 4. Record of the movements of the partly-filled stomach with a signal magnet tracing below indicating the egress of the gastric contents in drops through a duodenostomy situated just below the pyloric sphincter.

time of or immediately after the rise in intragastric pressure as shown at *a* in figure 3 and in a particularly pure form at *a* in figure 4. In the last-mentioned case the contractions of the stomach which result in this ejection of chyme are identical with the contractions of the so-called empty or nearly empty stomach which are responsible for the sensation recognized universally as hunger pangs. Now, hunger contractions are known to result from peristalses sweeping over the



stomach toward the pylorus. We have, therefore, no hesitation in stating that there is certain relation between the opening of the pylorus and particularly vigorous peristalses but that a moderate general rise in intragastric pressure (fig. 3, *b*) in the absence of powerful peristalsis may effect the same result. But we again point out that the graphic record of the motor activity of the stomach is quite deceptive; for, where the activity was simultaneously observed fluoroscopically we have noted powerful waves of peristalsis when the record showed only moderate tonus changes (tonus rhythm of Rogers and Hardt). Comparing the contractions shown in figure 2, those of figure 4 are decidedly more vigorous. It is the latter type which is particularly related to an opening of the sphincter.

#### DISCUSSION

The results of Ivy (16) obtained after and quite independently of us are strikingly confirmatory of the observations just recorded. In describing emptying time of water (with ground meat) from the stomach, Ivy (p. 427) writes:

In every dog it passed (i.e., the  $H_2O$ ) in single gushes at varying intervals of from 10 to 30 seconds, each gush delivering from 5 to 30 cc. of water. These gushes as to time seemed to occur in groups, e.g., several of 10 second intervals would occur, then several of 15 seconds, then several of 12 seconds, and between these there might be interposed one or two of 5 second or of 30 second intervals. There was evidence of rhythm.

Although Ivy was not interested in the cause of this rhythm he concludes that "the gushes could easily correspond to the occurrence of the peristaltic waves or stomach contractions, as reported by von Mering in 1893." Ample evidence submitted by us in this paper justifies the assumption.

We are anxious to record here a difficulty which interfered for some time with a study of this problem. We planned originally to make direct observations, with the aid of a Kirstein light, of the behavior of the pyloric sphincter by looking at the sphincter through a glass cannula placed into the duodenostomy. Such attempts were invariably followed by pronounced and long-continued pylorospasm and vomiting. Vomiting is elicited with the greatest ease by any irritation of the duodenum. If great care is not exercised in introducing the collecting tube through the duodenostomy, vomiting invariably occurs. Reflex emesis is certainly more readily elicited from mechanical irri-

tation of the duodenal mucous membrane near the pylorus than from simple irritation of the gastric mucosa. Salivation and retching precede the act.

The failure of the fluid contents to reach the intestine under these conditions results from a true pylorospasm because of the duodenal irritation. Wave after wave of gastric peristalses would pass over the stomach but no ejection of chyme would follow. A silver director found the pylorus so tightly constricted that considerable pressure had to be applied before the instrument would pass into the stomach. Failure of fluid under these conditions to reach the intestine might have been due partly to a leveling of the gradient as conceived by Alvarez (17). Direct inspection, however, showed the pyloric sphincter tightly constricted (pylorospasm) and not patulous.

#### CONCLUSIONS

Sufficient evidence has been presented in this paper on the basis of experimental work on man and dog that there is a correlation between marked motor activity of the stomach (either as tonus changes or peristalses) and an inhibition in tone of the pyloric sphincter. The work of other investigators is not at variance with these results. On the contrary, most investigators recognize that factors other than the presence of free acid in the stomach near the pyloric sphincter are related to an opening of that sphincter.

Under the especial conditions described in our experiments, the intragastric contents issuing from the duodenostomy were usually acid toward phenolphthalein but rarely showed presence of free acidity to dimethylamidoazobenzene or congo red. Ivy (16) similarly found the water to issue neutral from the stomach. Only later did the acidity rise. Under normal conditions, according to Alvarez (18), the stomach wall follows gradients of irritability, rhythmicity and latent period from cardia to pylorus. With a gradient higher in the body of the stomach than near the pylorus, the increased pressure coming from above effects an opening of the pylorus even before the free acidity has reached a concentration sufficient to assume chemical control of that sphincter. It seems probable that even under normal conditions the chemical control has been greatly over-emphasized to the exclusion of other possibilities.

## SUMMARY

1. Fluoroscopic examination of the stomach of man while recording simultaneously graphically the motor activity of the stomach shows that the pylorus opens for the ejection of chyme with arrival at the pylorus of powerful advancing rings of constriction aided possibly by a general increase in tone of the stomach musculature as a whole.

2. In dogs, the intragastric contents issue from a duodenostomy either *a*, during a marked rise in the intragastric pressure probably unaccompanied by peristaltic activity, or *b*, more commonly just at or after the peristaltic wave or waves passing over the stomach have effected their greatest increase in intragastric pressure.

3. There was certainly a greater relation between the muscular activity and the opening of the pylorus than between the latter and the reaction of the intragastric contents.

4. Vomiting is more easily induced by irritating duodenal mucosa than by an irritation of the gastric mucosa.

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## PHYSIOLOGICAL STUDIES ON PLANARIA

### II. OXYGEN CONSUMPTION IN RELATION TO REGENERATION

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The present paper deals with the rate of oxygen consumption of Planaria during the process of regeneration. The physiology of this process has already been investigated in this laboratory by other methods and certain conclusions regarding the metabolic rate during regeneration have been reached. Chief among these methods is the direct susceptibility method, which consists, as explained in greater detail in the preceding paper of this series (1), in observing the time of death of living materials in certain toxic solutions. We believe for reasons previously given (1) that the time of death in these solutions is a measure of metabolic rate. By means of this direct susceptibility method, Child (2) has already determined the following facts regarding the metabolic rate of Planaria during regeneration.

1. The susceptibility of pieces of Planaria within the first few hours after they are cut is greater than that of intact worms similar to those from which the pieces came. Hence the rate of oxygen consumption should be greater after section.

2. The susceptibility of the main portion of the pieces then gradually falls and reaches a minimum in about twelve hours after section. From this time through three to four days after section it is about like that of intact worms. However, the susceptibility of the cut surfaces remains higher than that of the rest of the piece during this period and hence the total oxygen consumption should also be higher than that of intact worms. However, the factor of starvation must be considered, since, as shown in the first paper (1), the oxygen consumption decreases during the early stages of starvation.

3. The susceptibility of the pieces (both old tissue and regenerating surfaces) then begins to rise and continues to rise until the end of the regeneration process. Completely regenerated pieces are thus much



more susceptible than the original intact worms. The oxygen consumption should likewise increase during regeneration and the oxygen consumption of the regenerated pieces should be considerably greater than that of the original intact worms.

All of these expectations have been completely realized in the experiments to be reported. At the same time in this laboratory, Miss Harriet Robbins has determined the carbon dioxide production during regeneration and has found that it runs parallel to the oxygen consumption and the general susceptibility.

The present experiments were performed upon twelve different lots of *Planaria dorotocephala*. Each lot was selected at random from the general laboratory stocks and consisted of medium sized worms, 15 to 20 mm. in length, although no effort was made to select individuals of the same size, as this was not essential for the purpose of the experiments. The heads were removed from the worms the day before the experiment was begun in order to eliminate movement. The oxygen consumption of each lot of decapitated worms was determined. The worms were then cut into small pieces and the oxygen consumption of these pieces again determined at various intervals after section until the process of regeneration was completed. After each such determination the worms were weighed, and the oxygen consumption per unit weight could then be calculated. In some cases the regenerated worms were fed in order to eliminate the factor of starvation. In all cases, after regeneration was complete, the regenerated parts were cut off and the oxygen consumption of those portions which corresponded to the original pieces tested separately.

The method of determining the rate of oxygen consumption and the method of weighing have been described in the previous communication (1), to which the reader is referred for details.

Details regarding each lot of worms and the data obtained upon them are given in the following description and accompanying tables. To save space, the data upon lots 1 to 6 are condensed in table 1, only the final calculations of the rate of oxygen consumption being presented. The data upon lots 7 to 12 are given in detail in tables 2 to 7. In these last mentioned tables, the time since section is stated in the first column, the actual cubic centimeters of oxygen consumed in a given time interval in the second column, the weight in grams in the third, and the oxygen consumed per unit weight per unit time in the fourth column. For the purposes of this calculation, purely arbitrary units of time and weight were selected, units, however, approximating those actually



encountered in the experiments. The time unit selected is two hours, and the unit of weight 0.5 gram; and the figures given in all of the columns of table 1 and in the fourth columns of tables 2 to 7 are therefore the cubic centimeters of oxygen consumed in two hours by 0.5 gram of Planaria.

TABLE 1

*Record of lots 1 to 6. Temperature 22°C.*

| CONDITION OF WORMS                                     | OXYGEN CONSUMED BY<br>0.5 GRAM IN 2 HOURS |       |       | CONDITION OF WORMS  | OXYGEN CONSUMED BY<br>0.5 GRAM IN 2 HOURS |       |       |
|--|---|-------|-------|---|---|-------|-------|
|  | Lot 1                                     | Lot 2 | Lot 3 |   | Lot 4                                     | Lot 5 | Lot 6 |
|  | cc.                                       | cc.   | cc.   |   | cc.                                       | cc.   | cc.   |
| Decapitated, intact, 2 days since feeding              | 0.30                                      | 0.29  | 0.28  | Decapitated, intact, 3 days since feeding                       | 0.26                                      | 0.27  | 0.26  |
| Cut into small pieces                                  |   |       |       | Cut into small pieces   |   |       |       |
| No to 8 hours after section                            | 0.35                                      | 0.34  | 0.32  | No to 3 hours after section                                     | 0.32                                      | 0.35  | 0.31  |
|  | 0.35                                      | 0.33  | 0.29  |   |   |       |       |
|  | 0.34                                      | 0.36  | 0.30  |   |   |       |       |
| 1 day after section                                    | 0.35                                      | 0.31  | 0.33  | 2 days after section  | 0.33                                      | 0.32  | 0.36  |
| 4 days after section                                   | 0.27                                      | 0.27  | 0.29  | 7 days after section  | 0.30                                      | 0.30  | 0.32  |
| 8 days after section                                   | 0.32                                      | 0.28  | 0.31  | 13 days after section   | 0.32                                      | 0.32  | 0.38  |
| 16 days after section                                  | 0.54                                      | 0.55  | 0.53  | 22 days after section   | 0.45                                      | 0.41  | 0.43  |
| Worms fed four times                                   |   |       |       | Regenerated parts cut off                                       |   |       |       |
| 23 days after section, 2 days since feeding            | 0.62                                      | 0.56  | 0.60  | 23 days after 1st section; few to 22 hours after second section | 0.36                                      | 0.37  | 0.37  |
| Regenerated parts cut off                              |   |       |       |   |   |       |       |
| 25 days since 1st section; 2 days since second section | 0.59                                      | 0.53  | 0.63  |   |   |       |       |

*Record of lots 1 to 3 (table 1).* The worms in these lots were collected on March 12, 1919; they were last fed on March 24; their heads were removed on March 25, and on March 26, the oxygen consumption of the decapitated worms was measured. It ranged in the three lots from 0.28 to 0.30 cc. of oxygen in two hours per

TABLE 2  
*Record of lot 7. Temperature 21°C.*

| CONDITION OF WORMS  | OXYGEN CONSUMED IN TEST               | WEIGHT | CALCULATION, AVERAGE AMOUNT OF OXYGEN CONSUMED BY 0.5 GRAM IN 2 HOURS |
|---|---------------------------------------|--------|---|
|   | cc.                                   | grams  | cc.   |
| Decapitated, intact, 1 day since feeding                        | 0.29 in 1½ hours<br>0.34 in 1½ hours* | 0.799  | 0.26  |
| <i>Cut into small pieces</i>                                    |                                       |        |   |
| 1 to 8 hours after section                                      | 0.35 in 1½ hours<br>0.40 in 1½ hours  | 0.762  | 0.32  |
| 20 to 22 hours after section                                    | 0.39 in 1½ hours                      | 0.673  | 0.38  |
| 2 days after section  | 0.36 in 1½ hours<br>0.35 in 1½ hours  | 0.628  | 0.37  |
| 7 days after section  | 0.20 in 1½ hours<br>0.23 in 1½ hours  | 0.501  | 0.28  |
| 15 days after section   | 0.32 in 2 hours<br>0.28 in 2 hours    | 0.363  | 0.41  |
| <i>Worms fed three times</i>                                    |                                       |        |   |
| 22 days after section; 1 day since feeding                      | 0.39 in 2 hours<br>0.38 in 2 hours    | 0.419  | 0.45  |
| <i>Regenerated parts cut off</i>                                |                                       |        |   |
| 23 days since first section; several hours since second section | 0.26 in 2 hours<br>0.34 in 2 hours    | 0.308  | 0.48  |

\* Whenever practicable, two separate determinations of the oxygen consumption were made on each occasion.

0.5 gram. The worms were then cut into small pieces and in several determinations taken during the first eight hours after section, the oxygen consumed ranged from 0.29 to 0.36 cc.—a distinct increase over the preceding figure. On the following day the oxygen consumption was about the same—0.31 to 0.35 cc. On March 31, four days after section, the oxygen consumption had fallen to 0.27 to

0.29 cc. On April 4, eight days after section, it was rising again, being 0.28 to 0.31; and on April 12, sixteen days after section, all of the pieces having undergone complete regeneration, the oxygen consumption was found to have increased greatly, having risen to 0.53 to 0.55 cc. The regenerated worms were now fed four times, on April 12, 13, 15 and 17, and on April 19 (the worms thus being in

TABLE 3  
*Record of lot 8, Temperature 21°C.*

| CONDITION OF WORMS  | OXYGEN CONSUMED IN TEST              | WEIGHT | CALCULATION, AVERAGE AMOUNT OF OXYGEN CONSUMED IN 2 HOURS BY 0.5 GRAM |
|---|--------------------------------------|--------|---|
|   | cc.                                  | grams  | cc.   |
| Decapitated, intact, one day since feeding                      | 0.37 in 1½ hours<br>0.39 in 1½ hours | 0.952  | 0.26  |
| Cut into small pieces   |                                      |        |   |
| 1 to 8 hours after section                                      | 0.40 in 1½ hours<br>0.46 in 1½ hours | 0.914  | 0.31  |
| 20 to 22 hours after section                                    | 0.48 in 1½ hours                     | 0.813  | 0.38  |
| 2 days after section  | 0.45 in 1½ hours<br>0.40 in 1½ hours | 0.755  | 0.37  |
| 7 days after section  | 0.25 in 1½ hours<br>0.24 in 1½ hours | 0.598  | 0.27  |
| 15 days after section   | 0.35 in 2 hours<br>0.33 in 2 hours   | 0.435  | 0.39  |
| Worms fed three times   |                                      |        |   |
| 22 days after section; 1 day since feeding                      | 0.48 in 2 hours<br>0.46 in 2 hours   | 0.502  | 0.46  |
| Regenerated parts cut off                                       |                                      |        |   |
| 23 days since first section; several hours since second section | 0.28 in 2 hours<br>0.33 in 2 hours   | 0.369  | 0.41  |

the same state regarding nutrition as the original lots), the oxygen consumption was found to be 0.56 to 0.62 cc., a slight increase. The regenerated tissue at both ends of the worms was now cut off and two days later, on April 21, the remaining portions, corresponding as nearly as possible to the original pieces, were tested. Their oxygen consumption was 0.53 to 0.63 cc. The temperature throughout the experiments was 22°C.  $\pm$  0.5.

*Record of lots 4 to 6 (table 1).* The worms in this lot were collected during the early winter of 1919, and were large and well-fed specimens. They were last fed on April 7, their heads cut off on April 9, and the decapitated worms tested on April 10. The oxygen consumption was 0.26 to 0.27 cc. They were then cut into small pieces and within the first few hours after section, their oxygen con-

TABLE 4  
*Record of lot 9. Temperature 21°C.*

| CONDITION OF WORMS  | OXYGEN CONSUMED IN TEST              | WEIGHT | CALCULATION, AVERAGE AMOUNT OF OXYGEN CONSUMED IN 2 HOURS BY 0.5 GRAM |
|---|--------------------------------------|--------|---|
|   | cc.                                  | grams  | cc.   |
| Decapitated, intact, one day since feeding                      | 0.33 in 1½ hours<br>0.35 in 1½ hours | 0.838  | 0.27  |
| Cut into small pieces   |                                      |        |   |
| ½ to several hours after section                                | 0.38 in 1½ hours<br>0.40 in 1½ hours | 0.790  | 0.33  |
| 21 to 23 hours after section                                    | 0.45 in 1½ hours                     | 0.714  | 0.42  |
| 2 days after section  | 0.39 in 1½ hours<br>0.38 in 1½ hours | 0.658  | 0.39  |
| 7 days after section  | 0.21 in 1½ hours<br>0.24 in 1½ hours | 0.530  | 0.28  |
| 15 days after section   | 0.32 in 2 hours<br>0.30 in 2 hours   | 0.375  | 0.41  |
| Worms fed three times   |                                      |        |   |
| 22 days after section; 1 day since feeding                      | 0.41 in 2 hours<br>0.41 in 2 hours   | 0.448  | 0.47  |
| Regenerated parts cut off                                       |                                      |        |   |
| 23 days since first section; several hours since second section | 0.36 in 2 hours<br>0.39 in 2 hours   | 0.338  | 0.55  |

sumption was found to have risen to 0.31 to 0.35 cc. Forty-eight to seventy-two hours after section, it was about the same, 0.32 to 0.36 cc. On April 17, seven days after section, it had fallen slightly to 0.30 to 0.32 cc. On April 23, thirteen days after section, it was rising again, being 0.32 to 0.38 cc.; and on May 2, twenty-two days after section, when the pieces had undergone complete regeneration, the

oxygen consumption was much higher than at the beginning of the experiment, being 0.41 to 0.45 cc. The regenerated portions were now removed and the oxygen consumption of the remaining pieces, corresponding to the original pieces, was 0.36 to 0.37 cc. The temperature throughout these experiments was also  $22^{\circ}\text{C.} \pm 0.5$ .

TABLE 5  
*Record of lot 10. Temperature  $18^{\circ}\text{C}$ .*

| CONDITION OF WORMS  | OXYGEN CONSUMED<br>IN TEST         | WEIGHT | CALCULATION,<br>AVERAGE<br>AMOUNT OF<br>OXYGEN<br>CONSUMED<br>IN 2<br>HOURS BY<br>0.5 GRAM |
|---|------------------------------------|--------|--|
|   | cc.                                | grams  | cc.  |
| Decapitated, intact, four days since feeding                | 0.23 in 2 hours<br>0.21 in 2 hours | 0.599  | 0.18   |
| Cut into small pieces                                       |                                    |        |  |
| $1\frac{1}{2}$ to $3\frac{1}{2}$ hours after section        | 0.29 in 2 hours                    | 0.512  | 0.28   |
| 18 to 22 hours after section                                | 0.29 in 2 hours<br>0.23 in 2 hours | 0.469  | 0.27   |
| 2 days after section  | 0.23 in 2 hours<br>0.20 in 2 hours | 0.452  | 0.23   |
| 4 days after section  | 0.18 in 2 hours                    | 0.403  | 0.22   |
| 8 days after section  | 0.17 in 2 hours<br>0.17 in 2 hours | 0.332  | 0.25   |
| 17 days after section                                       | 0.15 in 2 hours<br>0.16 in 2 hours | 0.181  | 0.42   |
| Regenerated parts cut off                                   |                                    |        |  |
| 22 days since first section; few hours since second section | 0.05 in 2 hours<br>0.06 in 2 hours | 0.113  | 0.24   |

*Record of lots 7 to 9 (tables 2 to 4).* The stock from which these worms were taken was collected in January, 1919. They were last fed on April 23, their heads removed on the same day, and their rate of oxygen consumption tested on April 24. They were then cut into small pieces and their rate of oxygen consumption tested at various intervals after section, as in the preceding experiments. As before, the oxygen consumption was found to be greater during the first few hours



up through two days after section than it was in the same worms before section; it then fell, as shown by a measurement on May 1, seven days after section. From this time on, the oxygen consumption rose and on May 9, fifteen days after section, it was considerably higher than that of the original intact worms. The regenerated worms were then fed on May 10, 12 and 14, and the oxygen consump-

TABLE 6  
*Record of lot 11. Temperature 18°C.*

| CONDITION OF WORMS  | OXYGEN CONSUMED<br>IN TEST                | WEIGHT         | CALCULATION,<br>AVERAGE<br>AMOUNT OF<br>OXYGEN<br>CONSUMED<br>IN 2<br>HOURS BY<br>0.5 GRAM |
|---|---|----------------|--|
|   |   |                | cc.  |
| Decapitated, intact, four days since feeding                | cc.<br>0.23 in 2 hours<br>0.24 in 2 hours | grams<br>0.560 | 0.21   |
| Cut into small pieces                                       |   |                |  |
| 1 to 3 hours after section                                  | 0.29 in 2 hours                           | 0.540          | 0.26   |
| 18 to 22 hours after section                                | 0.29 in 2 hours<br>0.25 in 2 hours        | 0.503          | 0.26   |
| 2 days after section  | 0.25 in 2 hours<br>0.23 in 2 hours        | 0.476          | 0.25   |
| 4 days after section  | 0.20 in 2 hours                           | 0.430          | 0.23   |
| 8 days after section  | 0.20 in 2 hours<br>0.19 in 2 hours        | 0.360          | 0.27   |
| 17 days after section                                       | 0.17 in 2 hours<br>0.15 in 2 hours        | 0.153          | 0.52   |
| Regenerated parts cut off                                   |   |                |  |
| 22 days since first section; few hours since second section | 0.06 in 2 hours<br>0.05 in 2 hours        | 0.085          | 0.32   |

tion tested on May 15, one day after feeding, as was the case with the original lots. As before, feeding resulted in a distinct increase in the rate of oxygen consumption. On May 16, the regenerated tissue was removed, and the oxygen consumption of the remaining pieces, corresponding to the original pieces, was again tested. The temperature throughout was 21°C.  $\pm$  0.5.

*Record of lots 10 to 12 (tables 5 to 7).* The members of lots 10 and 11 came from a stock which had been for some time in the laboratory (date of collection not known). They were large, well-fed individuals. The worms in lot 12 came from a general mixed stock containing material which had been used for other experimental purposes. The three lots were last fed on May 2, their heads were removed

TABLE 7  
*Record of lot 12. Temperature 18°C.*

| CONDITION OF WORMS                                      | OXYGEN CONSUMED<br>IN TEST         | WEIGHT | CALCULATION,<br>AVERAGE<br>AMOUNT OF<br>OXYGEN<br>CONSUMED<br>IN 2<br>HOURS BY<br>0.5 GRAM |
|---|------------------------------------|--------|--|
|   |                                    |        |  |
|   | cc.                                | grams  | cc.  |
| Decapitated, intact, four days since feeding            | 0.23 in 2 hours<br>0.24 in 2 hours | 0.681  | 0.17   |
| Cut into small pieces                                   |                                    |        |  |
| $\frac{1}{2}$ to $2\frac{1}{2}$ hours after section     | 0.30 in 2 hours                    | 0.615  | 0.24   |
| 18 to 22 hours after section                            | 0.31 in 2 hours<br>0.22 in 2 hours | 0.566  | 0.23   |
| 2 days after section                                    | 0.28 in 2 hours<br>0.27 in 2 hours | 0.532  | 0.25   |
| 4 days after section                                    | 0.22 in 2 hours                    | 0.499  | 0.22   |
| 8 days after section                                    | 0.21 in 2 hours<br>0.21 in 2 hours | 0.422  | 0.24   |
| 17 days after section                                   | 0.18 in 2 hours<br>0.20 in 2 hours | 0.263  | 0.36   |
| Regenerated parts cut off                               |                                    |        |  |
| 22 days since first section; 1 day since second section | 0.11 in 2 hours<br>0.10 in 2 hours | 0.166  | 0.31   |

on May 5, and the oxygen consumption of the decapitated worms was tested on May 6. The worms were then cut into small pieces, and the oxygen consumption of the pieces tested at intervals after section as in the preceding experiments. The rate of oxygen consumption was again found to be greater after section than before, remaining high through two days after section, then falling, and finally rising as regeneration was completed. The regenerated parts were then removed,

and the rate of oxygen consumption of the parts corresponding to the original pieces tested. The temperature throughout these three experiments was  $18^{\circ}\text{C}.$   $\pm 0.5$ .

A word is required regarding the degree of movement of the worms during these experiments, since movement increases the rate of oxygen consumption. The decapitated whole worms with which the experiments are begun are always perfectly quiet, as are also the pieces cut from them during the first ten or twelve hours after section. Twenty-four hours after section and from this time on through the greater part of the regeneration process there is some slight movement among the pieces but this is not sufficient to affect the measurements of oxygen consumption. The completely regenerated worms, however, usually move about considerably, and the figures obtained at the end of the regeneration process are probably slightly too high on this account, although the amount of movement was reduced in most cases by shading the worms and placing them in the flasks in which they were to be tested some time before the test was carried out. In experiments 10 to 12 inclusive, movement was practically completely eliminated in all cases by testing the worms at a temperature of  $18^{\circ}\text{C}.$  Since the results in these three experiments do not differ from those of the other nine, it is reasonably certain that movement is never a significant factor in the general result. After the regenerated worms are fed, they remain perfectly quiet, and the pieces obtained by cutting off the regenerated regions are also motionless.

#### CONCLUSIONS AND DISCUSSION

The data upon twelve different lots of worms are in accord with each other, and justify the following conclusions:

1. The oxygen consumption per unit time per unit weight of a given lot of worms is greater after these worms have been cut into small pieces than it was while they were intact. Section therefore increases the rate of oxygen consumption. The same conclusion had already been reached in this laboratory by means of other methods. Thus Child (2) long ago observed that the susceptibility of newly-cut pieces of *Planaria* to cyanide and other toxic solutions is much greater than that of intact worms. This increase in susceptibility is greatest at the cut surfaces but involves the entire piece also, in the case of small pieces. In long pieces the increased susceptibility is observable only at the cut surfaces and adjacent regions. This demonstrates that the

increase in susceptibility and rate of oxygen consumption following section are due primarily to the injury of cutting and spread from the cut surfaces with a decrement to the remainder of the pieces. The phenomenon is indeed only one example of the general physiological fact that injury is a form of stimulation, expressing itself in an increased rate of respiratory exchange, increased production of metabolic products, and electrical negativity.

The increase in susceptibility after section has been observed in this laboratory not only in *Planaria* but in a variety of the lower organisms. I found it to be true for several species of small fresh-water annelids (3) and incidentally in the course of numerous investigations upon the susceptibility of the lower forms, we have invariably observed that injured places are more susceptible than adjacent uninjured regions. Scott (4) noted that the oxygen consumption of the sea-anemone *Sagartia* is increased after section.

Not only are the rate of oxygen consumption and the susceptibility to toxic solutions of pieces of *Planaria* increased after section but, as Child (2) has shown with the aid of the Tashiro biometer, the carbon dioxide production is likewise accelerated. Recently Miss Robbins has repeated and extended these observations using the phenolsulphonephthalein method of Haas (5). Tashiro in his book *A Chemical Sign of Life* has shown that such an acceleration of carbon dioxide production as a consequence of injury is a practically universal biological phenomenon and he suggests that the occurrence of this change may be regarded as a proof that the material in question is living.

2. The increased rate of oxygen consumption following section was observed in these experiments to continue in most of the lots through forty-eight hours after section. Tests by the susceptibility method show that the susceptibility of the middle portions of the piece gradually falls after section and has returned to the condition found in intact worms in about twelve hours. However, the susceptibility of the cut and regenerating surfaces always remains higher than that of the middle part of the pieces. Hence the oxygen consumption, since it includes all parts of the pieces, must be greater than that of intact worms during this early part of the regeneration period. Nevertheless, it was expected that the oxygen consumption twenty-four and forty-eight hours after section would be somewhat less than it was within the first few hours after section. In lots 2, 5 and 10 this was the case but not in the other nine lots. It is therefore evident that extraneous factors enter into the determinations during this period, and this is further



indicated by the greater variability in the figures obtained during the first forty-eight hours after section than at later periods. It seems highly probable that the frequent weighings and handling during this period acted as sources of stimulation. This is further rendered probable by the greater uniformity in the measurements obtained with lots 10 to 12 which were tested at a lower temperature in order to eliminate some of these factors.

3. During the period from three or four days to a week after section the oxygen consumption is falling. Since the susceptibility method shows no such fall in metabolic rate during this time, the decrease must be regarded as due entirely to starvation. As shown in the first paper of this series (1), the rate of oxygen consumption decreases continuously during the first two weeks of starvation, this resulting from lack of activity of the digestive tract. In the case of regenerating pieces the total oxygen consumption does not decrease as a result of starvation to as great an extent as in non-regenerating worms, since this decrease is partially compensated by the increased metabolic rate of the regenerating ends of the pieces. The total oxygen consumption, therefore, of the pieces during the period from three or four days to a week after section is the resultant of two conditions, an increase due to regeneration and a decrease due to starvation. During this period the latter factor predominates.

4. From a week after section to the completion of the process of regeneration the rate of oxygen consumption is continually increasing. The oxygen consumption of the completely regenerated worms is very much greater than that of the intact worms from which the pieces were taken. The amount of this increase ranges in the twelve lots of worms from 50 to 150 per cent. Regeneration is thus a method of increasing the metabolic rate of *Planaria*. This conclusion had already been reached in this laboratory for *Planaria* and other forms through the use of the susceptibility method and further for *Planaria* through a study of carbon dioxide production during regeneration. Scott (4) found that the rate of oxygen consumption of a sea-anemone is increased by regeneration.

The objection may be raised that the increase in rate of oxygen consumption per unit weight during regeneration may be only apparent since the decrease in weight might be due to a loss of non-respiring materials. But if this were the case, the oxygen consumption should increase during the early stages of regeneration, when the weight is decreasing most rapidly. As a matter of fact however, both in the



present series of experiments and in the experiments on starvation previously reported, the oxygen consumption is also decreasing during this time. In the later stages of both regeneration and starvation, the loss of weight occurs at about a uniform rate or even decreases slightly; yet during this time the rate of oxygen consumption is continually rising. It is therefore reasonably certain that the observed acceleration of oxygen consumption in regeneration and starvation represents a real increase in the basic metabolism of the cells of the organism. The same conclusion was reached by Benedict (6) in his study of the metabolism of man during prolonged fasting.

The present experiments therefore support Child's conception of regeneration as a method of bringing about rejuvenescence—that is, restoring the organism to a metabolic condition comparable to that of young animals.

5. The regenerated worms of lots 1, 2, 3, 7, 8 and 9 were fed in order to bring them to a state of nutrition comparable to that of the original pieces, since, as already explained, starvation tends to lower the rate of oxygen consumption during the period in which the pieces are regenerating. In all six cases, the rate of oxygen consumption of the regenerated worms was increased by feeding. The tests were of course made at the same time interval after the last feeding as had been the case with the original pieces. It is naturally impossible to assert that after three or four feedings the regenerated pieces are in the same state of nutrition as the original pieces from which they came but at least they are as the result of such feeding more comparable to the latter. It is only after the effect of starvation has been eliminated that the real extent of the rise in oxygen consumption resulting from regeneration can be detected.

6. The rise in rate of oxygen consumption due to regeneration involves not only the newly regenerated portions, but also the old tissue of the piece. This was determined by removing and discarding the new heads and tails and testing the rate of oxygen consumption of the pieces remaining after this operation, such pieces corresponding as nearly as possible to the original pieces. Presumably these pieces are stimulated by section as were the original pieces although we cannot say whether they are stimulated to the same degree through the removal of the regenerated portions as are pieces cut from whole worms, as this point has not yet been subjected to experimental test. It is probable that they are less stimulated by section than are pieces cut from whole worms since in newly regenerated worms the old portions

have not yet established connections with the newly formed ends. Probably the severance of morphological and physiological connections and conduction paths is one of the chief factors in the stimulation observable after cutting.

Since, however, we have no definite information upon the degree of stimulation by section in pieces cut from regenerated worms, it is necessary to assume that it is the same as that in pieces cut from individuals not recently regenerating. One must therefore compare these pieces comprising the old portions of the regenerated worms with the original pieces at approximately the same lengths of time after section. The necessary data for this comparison are given in the tables. It is evident that when the regenerated worms were fed, the oxygen consumption of the old portions cut from them is always considerably higher than that of the original pieces the same length of time after section and after feeding. When the regenerated worms were not fed, the oxygen consumption of the old tissue is less but in all cases, except in lot 10, it is still higher than that of the original pieces considered the same length of time after section. This one exception may be due to variability in the degree of starvation.

It is therefore certain that when restored to the same condition of nutrition, pieces from which regenerated tissue has grown out have a higher rate of oxygen consumption than the same pieces before such growth occurred.

It is further evident from the data at hand that the rate of oxygen consumption of the old portions of the regenerated worms is less than that of the regenerated regions since in all cases where the worms were not fed, the oxygen consumption is reduced by cutting off the regenerated tissue. Where the worms were fed, a comparison cannot be made since the rate of oxygen consumption after feeding depends upon the number of worms which feed and upon the amount of food which they ingest, and this in return depends upon the morphological condition of the digestive tract, a factor which is very variable in a mixed lot of regenerating worms such as those used in these experiments.

#### SUMMARY

1. The oxygen consumption of a given lot of *Planaria* per unit weight is increased when they are cut into small pieces. This increase is due to the stimulation of injury.

2. This increase persists through about forty-eight hours after cutting but may fall slightly during this period. The persistence of the

increase is associated with the activity of the cut surfaces, the original tissue in the pieces probably not being involved.

3. The oxygen consumption of the pieces then falls and remains at a low level for about one week. This fall is due entirely to starvation. The fact, however, that the decrease in rate of oxygen consumption during this period is not as great as in starving non-regenerating worms indicates that the oxygen consumption of the regenerating regions has remained high throughout.

4. The oxygen consumption then begins to rise and continues to rise as regeneration proceeds. The oxygen consumption of the completely regenerated worms is 50 to 150 per cent greater than was that of the worms from which the pieces were taken.

5. When the regenerated worms are fed in order to eliminate the factor of starvation their oxygen consumption rises to a still higher figure.

6. The increased rate of oxygen consumption in regenerated worms is due not only to the high metabolic rate of the regenerated tissue but also in part to an acceleration of the rate of oxygen consumption of the old tissue comprising the original pieces. In other words, when a piece of *Planaria* undergoes regeneration, its metabolic rate is thereby accelerated; its rate is not, however, as high as that of the regenerated regions.

7. These experiments confirm the conclusion already reached by Child through other methods that the process of regeneration is a rejuvenating process, restoring the organism to a metabolic condition comparable to that of young organisms.

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## EFFECT OF ANESTHESIA AND OPERATION ON CERTAIN METABOLITES

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A previous study of anesthesia demonstrated certain changes in the acid-base equilibrium during that process (1). For practical needs in the matter, the question of whether to give carbon dioxide with the anesthetic or alkali before or after the anesthetic, was continually the basis of the work. A certain few surgical patients show uncompensated acidosis after anesthesia and operation. These studies were to show, if possible, changes in the nitrogen metabolism.

### TECHNIQUE

Blood was collected before anesthesia was begun, and after the anesthetic was stopped, from the arm veins of unselected surgical patients admitted to the Lankenau Hospital, and operated in the clinic of Dr. John B. Deaver. Oxalate was used to prevent clotting. Blood-urea was determined by the urease method. Non-protein nitrogen was determined on 10 cc. of blood; coagulation of the proteins was accomplished by trichloroacetic acid, and nitrogen estimated on the filtrate by the Kjeldahl method. Urine in the first series was collected twenty-four hours before, and twenty-four hours after operation; and in later experiments, it was collected at shorter intervals. The results were exactly the same. Urea was determined by the urease and titration method; ammonia by aeration and titration; phosphates by the uranium titration method; total acidity by titration with deci-normal sodium hydroxide, using phenolphthalein as indicator to the first faint pink.

Our a priori arguments were as follows: If acids are increased during anesthesia, the titrable acidity of the urine should increase. The blood-urea should diminish as a result of increased demand for ammonia. The urine urea should possibly decrease and the urine



ammonia increase. The figures shown in the table express the results of determinations on about ninety patients. The urinary acidity was increased very definitely in every case until in some instances, almost half normal acid was excreted. The blood-urea and non-protein nitrogen were increased in every case. In the urine the general tendency of the ammonia was to increase, whereas the excretion of urea was either increased or diminished, with apparently no relation between the urea in the urine and in the blood. The question of retention or increased production immediately suggested itself in the case of the increase in the blood-urea and non-protein nitrogen.

TABLE 1

| BLOOD                            |       |                  |       | URINE   |          |       |          |        |          |       |          |                  |          |       |          |
|----------------------------------|-------|------------------|-------|---------|----------|-------|----------|--------|----------|-------|----------|------------------|----------|-------|----------|
| Non-protein nitrogen per 100 cc. |       | Urea per 100 cc. |       | Ammonia |          |       |          | Urea   |          |       |          | Titrable acidity |          |       |          |
| Before                           | After | Before           | After | Before  |          | After |          | Before |          | After |          | Before           |          | After |          |
| mgm.                             | mgm.  | mgm.             | mgm.  | gm.     | per cent | gm.   | per cent | gm.    | per cent | gm.   | per cent | cc.              | per cent | cc.   | per cent |
| 40.4*                            | 37.8  | 35               | 43    | 0.150   | 0.097    | 0.121 | 0.060    | 2.21   | 1.43     | 1.93  | 0.97     | 106.0            | 69       | 302   | 101      |
| 23.0                             | 40.0  | 13               | 20    | 0.006   | 0.007    | 0.045 | 0.043    | 0.36   | 0.41     | 1.30  | 0.92     | 1.7              | 2        | 176   | 126      |
| 33.6*                            | 21.5  | 13               | 22    | 0.073   | 0.048    | 0.039 | 0.060    | 1.62   | 1.06     | 0.47  | 0.73     | 79.0             | 55       | 87    | 134      |
| 42.8                             | 44.5  | 24               | 34    | 0.166   | 0.070    | 0.365 | 0.121    | 3.27   | 1.39     | 5.17  | 1.72     | 42.0             | 18       | 498   | 166      |
| 44.2                             | 48.1  | 22               | 27    | 0.026   | 0.052    | 0.047 | 0.047    | 0.43   | 0.87     | 0.57  | 0.57     | 29.0             | 58       | 80    | 80       |
| 29.9                             | 34.4  | 12               | 16    | 0.022   | 0.019    | 0.041 | 0.045    | 1.45   | 1.25     | 0.72  | 0.80     | 19.0             | 17       | 83    | 93       |
| 36.9                             | 43.6  | 11               | 30    | 0.020   | 0.081    | 0.268 | 0.063    | 0.59   | 1.74     | 7.35  | 1.73     | 14.0             | 40       | 408   | 115      |
| 44.2                             | 49.2  | 13               | 38    | 0.232   | 0.082    | 0.016 | 0.055    | 3.96   | 1.41     | 0.35  | 1.19     | 112.0            | 40       | 3     | 110      |
| 35.8                             | 48.1  | 12               | 15    | 0.084   | 0.030    | 0.240 | 0.092    | 4.11   | 1.46     | 3.40  | 1.41     | 47.0             | 17       | 325   | 125      |
| 39.4                             | 44.8  | 25               | 45    | 0.053   | 0.070    | 0.094 | 0.049    | 0.66   | 0.88     | 0.89  | 0.47     | 16.0             | 22       | 83    | 44       |

\* These were the only cases in the series which decreased.

MacNider's excellent work has shown that during anesthesia, in a non-nephropathic animal, the response to diuretics is approximately normal (2). From the fact that the blood-urea was universally increased, and the fact that among these patients there were numbers whose kidneys were non-nephropathic, as far as it was possible to learn, we can say that if retention does play a part, it does not tell the whole story. Therefore, there must have been an increased metabolism in the usual way down to urea. There must, however, also have been some slight relative changes in the nitrogen metabolism; for the increases in the non-protein nitrogen do not entirely parallel the increases



in blood-urea. As regards the urinary findings, the urea excretion bears no constant relationship to the variations in the blood-urea. This is in harmony with observations of urea excretion and blood-urea levels in other circumstances. We are not convinced that any definite and strict mathematical relationship has been shown as yet. That the ammonia is not increased in each case is perhaps not as surprising as first thought would suggest. A markedly increased elimination of phosphates accounts for some of the acidity of the urine (3). These no doubt play a part, as well as the sodium bicarbonate, in neutralizing the increased acids. In any given case, then, alkali is available in a number of forms, and in all probability, could a complete analysis be

TABLE 2  
*Urinary elimination of phosphates ( $P_2O_5$ )*

| BEFORE          | AFTER           |
|-----------------|-----------------|
| <i>per cent</i> | <i>per cent</i> |
| 0.89            | 1.2             |
| 0.304           | 1.1             |
| 0.392           | 0.922           |
| 0.25            | 0.865           |
| 0.09            | 0.46            |
| 0.31            | 0.732           |

made of all the acids and alkalies, both in the blood and urine, before and after anesthesia, a mathematical proportion between them could be worked out.

The possibility has been kept in mind that the changes in metabolism may not be altogether along the normal route. Further investigations, particularly directed to a search for abnormal intermediate products, as well as, perhaps, the products of an entirely abnormal metabolism,—those due, for example, to direct destruction of cytoplasm or nuclear material by the anesthetics,—must be conducted to clear up some points. This was afieid of our practical needs in the matter, and we have not carried them out.

#### SUMMARY

It has been demonstrated that small but very definite changes in metabolism take place during anesthesia. The reduction in bicarbonate of the blood plasma which occurs in that case must be due to

these changes, and not to mere over-ventilation, as suggested by Y. Henderson (4). Therefore, giving carbon dioxide with the anesthetic is contraindicated. Alkali should be given in selected cases.

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## EXPERIMENTAL SURGICAL SHOCK

### V. THE TREATMENT OF THE CONDITION OF LOW BLOOD PRESSURE WHICH FOLLOWS EXPOSURE OF THE ABDOMINAL VISCERA<sup>1</sup>

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This work was undertaken for the purpose of investigating under standard experimental conditions all the more important methods of treating a condition which exhibits the clinical signs of surgical shock in order to determine the relative value of the methods. While numerous such investigations have been made, only a portion of the entire field has been covered by a single investigation. Since in the different series of experiments various methods were used to produce the condition called shock it is impossible to compare the therapeutic results.

The method of producing the signs of shock was the same throughout in the present investigation and while the results may not be applied directly to all cases clinically diagnosed as surgical shock, the different methods of treating a condition which presents a common symptomatology can be accurately compared. Since it is obvious that the condition which the surgeon terms shock is due to a variety of causes, it is useless to attempt to find a specific therapeutic procedure, but as the symptoms are usually the same it is reasonable to suppose that some general therapeutic measures will be found.

The method of producing shock has varied greatly with the different investigations. In my work exposure of the abdominal viscera has seemed to afford the nearest approach to the production of shock presenting all the clinical signs (17). Our routine method was as follows: The animal, a dog, which had been fasted for from twelve

<sup>1</sup> Presented before the American Physiological Society, April, 1919, Baltimore.

to eighteen hours, was etherized in a closed cabinet, incubated, and a constant surgical anesthesia maintained by means of a Connell apparatus. Carotid blood pressure was recorded by means of a mercury manometer; sometimes the membrane manometer was also employed. After a record of normal blood pressure was obtained, the abdomen was opened and the viscera exposed. The only trauma to which the exposed viscera were subjected was the occasional gentle sponging with dry gauze or changing them from one side of the body to the other. When blood pressure had decreased and remained rather stationary at the desired level, which usually occurred about one to two hours after the exposure of the viscera, they were returned to the abdominal cavity and the wound was closed. After a length of time sufficient definitely to determine that blood pressure did not increase, procedures to improve the condition of the animal were instituted. The blood pressure was taken as a criterion of the animal's condition because it affords the easiest method of comparison. All other clinical signs of shock were also noted.

The maintenance of a constant anesthetic throughout the experiment removed the possibility of an error either in the interpretation of blood pressure records or in the general condition of the animal (19), (20). Anesthetic control experiments were carefully performed, the etherization being maintained at the same tension and for a length of time equal to that of the shock experiments. Practical conclusions can only be drawn from the results which apply to the condition in which the signs of shock were produced by exposure of the abdominal viscera, although it would seem that they should also be of value in a condition of a lowered and progressively decreasing blood pressure. The experiments were acute because it seemed that the therapeutic procedure would be put to a greater test if the animal was maintained under an anesthetic throughout the experiment and because the character of the experiment would not warrant the complete withdrawal of the anesthetic.

The fact should be emphasized that blood pressure which has been decreased and remains stationary below a certain level, and is allowed to remain there even for a very short time, is never restored and maintained by any known method of treatment. We have estimated the pressure below which no hope for restoration could be held as half the initial pressure maintained constant for one hour. In our experiments the methods of treatment, with very few exceptions, proved of no permanent value if the blood pressure had been decreased to less



Fig. 1. Kymograph record showing the successful use of acacia. *Record I*, normal blood pressure 118. *Record II*, after the abdominal viscera had been exposed 1½ hours; blood pressure 70. *Record III*, taken 30 minutes after the viscera were replaced; blood pressure 74; 160 cc. (20 cc. per kgm.) of a 6 per cent solution of acacia in 0.9 per cent sodium chlorid solution were injected (signal A-B); the injection was probably too rapid. The blood pressure increased to a maximum of 84. *Records IV, V, VI, VII and VIII* were taken at succeeding hours after the injection. The blood pressure was 84, 90, 95, 95 and 96 respectively. This is one of the few successful results following the use of acacia in the series of experiments.

Fig. 2. Kymograph record showing the favorable action of acacia. *Record I*, normal blood pressure 105. *Record II*, after 1 hour of exposure of the abdominal viscera; blood pressure 70. *Record III*, 15 minutes after replacing the viscera; blood pressure 68; 100 cc. (20 cc. per kgm.) of a 6 per cent acacia solution in 0.9 per cent sodium chlorid were injected slowly; blood pressure increased to a maximum of 82. *Record IV*, taken 30 minutes after injection; blood pressure 76. *Record V*, taken 1 hour after injection; blood pressure 80. *Records VI, VII, VIII and IX* were taken at successive hours after the injection with blood pressure of 92, 90, 98 and 85 respectively. These experiments seem to show that the result is as good as can be hoped for with acacia.

Fig. 3. Kymograph record showing the results of the injection of an alkaline acacia solution. *Record I*, normal blood pressure 148. *Record II*, after exposure of the abdominal viscera for 1 hour; blood pressure 88. *Record III*, after exposure of the abdominal viscera for 2 hours; blood pressure 75. *Record IV*, after replacing the viscera for 10 minutes and the injection of 146 cc. (20 cc. per kgm.) of a 7 per cent solution of acacia and 4 per cent solution sodium bicarbonate; the blood pressure increased to a maximum of 118. The succeeding records were taken at one-hour intervals after injection. The decrease in blood pressure occurring in the last records is characteristic of the action of an alkaline acacia solution, but usually blood pressure is not maintained for so long a time.

Fig. 4. Kymograph record showing a failure of acacia solution followed by a success with gelatine solution (Hogan's). *Record I*, normal blood pressure 140. *Record II*, 1½ hours after exposure of the abdominal viscera; blood pressure 82. *Record III*, 30 minutes after the replacing of the viscera; blood pressure 94; 104 cc. (20 cc. per kgm.) of a 6 per cent solution of acacia in 0.9 per cent sodium chlorid were injected (signal A-B). The injection may possibly have been made too rapidly; at any rate there was little improvement in blood pressure. *Record IV*, taken 35 minutes after the injection of acacia; blood pressure 70; 104 cc. of gelatine solution (Hogan's) were injected at the same rate the acacia had been injected; blood pressure increased to 112. *Records V, VI, VII and VIII* were taken at succeeding hours after the last injection. The blood pressure was respectively 100, 112, 118 and 110. The gelatine produced a much better result than acacia.

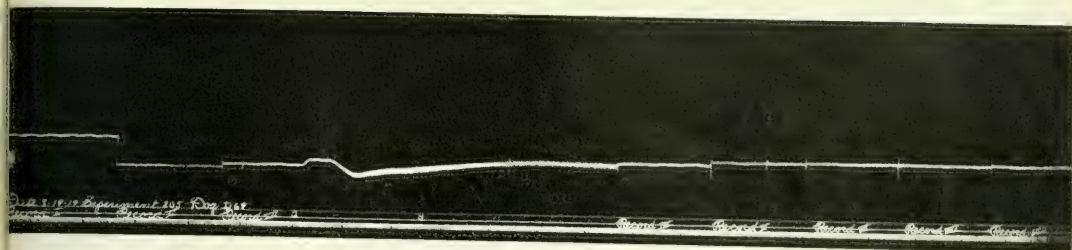


Fig. 1

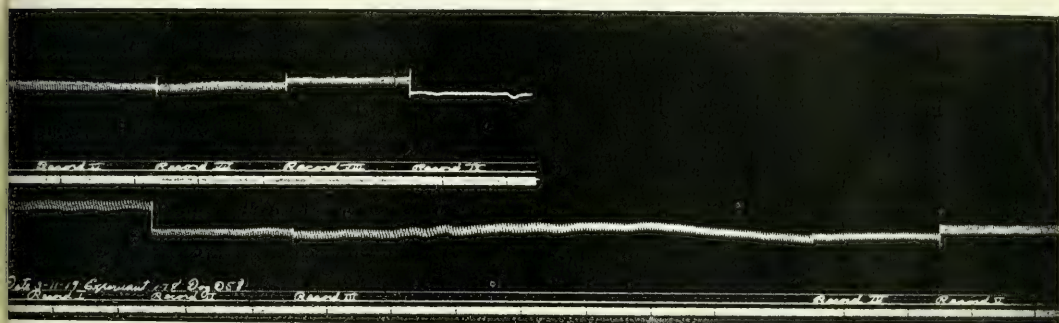


Fig. 2

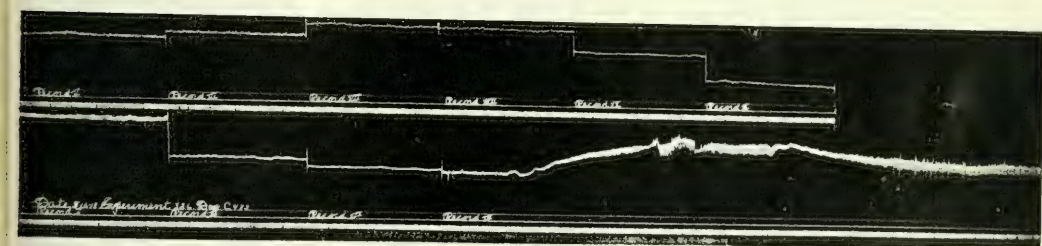


Fig. 3

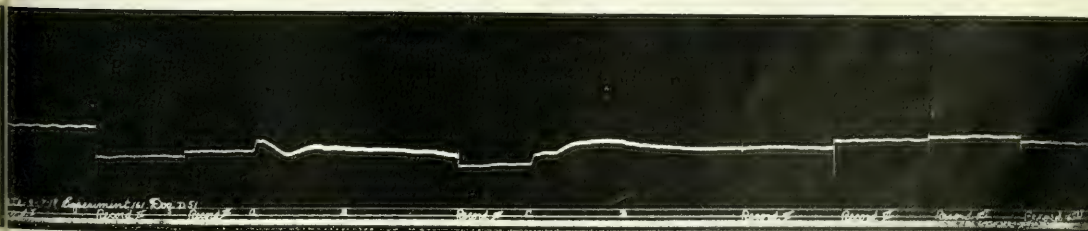


Fig. 4

Fig. 5. Kymograph record showing the action of the citrated blood after a failure of acacia and modified acacia solution. *Record I*, normal blood pressure 112. *Record II*, after exposure of the abdominal viscera for 1 hour; blood pressure 52. *Record III*, after the replacing of the viscera for 5 minutes; 70 cc. (20 cc. per kgm.) of a 6 per cent solution of acacia in 0.9 per cent sodium chlorid solution were injected (signal A-B). The blood pressure increased to a maximum of 80, but soon began to decrease and in 30 minutes it was 55. *Record IV*, taken 40 minutes after *record III*; blood pressure 50; 70 cc. of a modified acacia solution (6 per cent acacia, 10 per cent glucose, 1 per cent sodium carbonate, 1 per cent sodium sulphate) were injected (signal C-D). The blood pressure increased to a maximum of 110, but soon began to decrease. *Record V*, taken 40 minutes after *record IV*; blood pressure 60. *Record VI*, taken 1 hour after the last injection; blood pressure 56; 70 cc. of citrated blood were injected (signal E-F). The blood pressure increased to a maximum of 85. *Records VII, VIII, IX and X* were taken at succeeding hours after the last injection. Note the beneficial action of blood.

Fig. 6. Kymograph record showing the restoration and maintenance of blood pressure by the injection of blood after a failure of acacia solution. *Record I*, normal blood pressure 105. *Record II*, 1 hour after exposure of the abdominal viscera; blood pressure 70. *Record III*, immediately after the replacing of the viscera; blood pressure 40; 70 cc. (20 cc. per kgm.) of a 6 per cent acacia solution in 0.9 per cent sodium chlorid solution were injected (signal A-B). The blood pressure increased to a maximum of 70, but within 30 minutes had decreased to 50. *Record IV*, taken 50 minutes after *record III*; blood pressure 48, 70 cc. citrated blood were injected (signal C-D). The blood pressure increased to a maximum of 86. *Records V, VI and VII* were taken at succeeding hours after the last injection, with blood pressures of 88, 90 and 88, respectively.

Fig. 7. Kymograph record showing the effect of injection of dextrin followed by gelatine solution. *Record I*, normal blood pressure 115. *Record II*, 1 hour after the exposure of the abdominal viscera; blood pressure 65. *Record III*, 15 minutes after the replacing of the viscera; 150 cc. (20 cc. per kgm.) of a 20 per cent dextrin solution were injected. The blood pressure increased to a maximum of 120, but soon decreased to 60. *Record IV*, taken 1 hour after *record III*; blood pressure 58; 150 cc. gelatine solution (Hogan's) were injected; blood pressure increased to a maximum of 82. The succeeding records were taken at one-hour intervals after injection. Note the slow recovery and the failure of blood pressure.

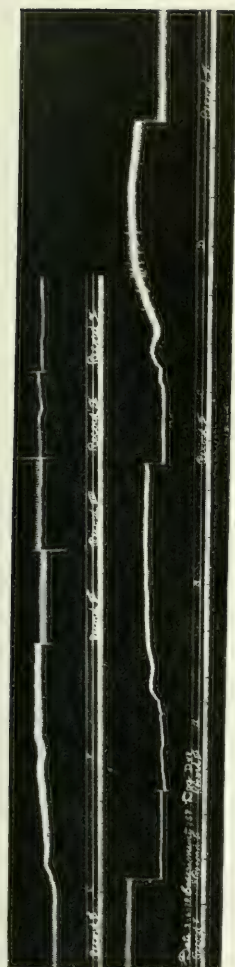


Fig. 5



Fig. 6

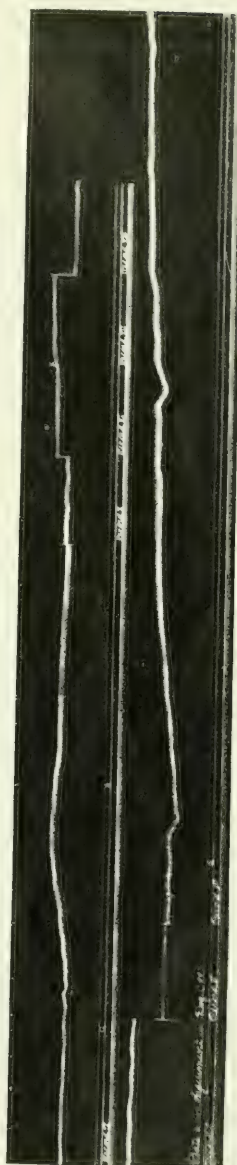


Fig. 7



Fig. 8. Kymograph record showing the results of the injection of dog serum. *Record I*, normal blood pressure 110. *Record II*, after exposure of the abdominal viscera for 1 hour; blood pressure 64. *Record III*, after the replacing of the viscera for 15 minutes; 110 cc. (20 cc. per kgm.) of dog serum were injected. The blood pressure was increased to a maximum of 110. The succeeding records, *IV* to *VIII*, were taken at one-hour intervals after injection. The serum restored blood pressure to normal and maintained it for 5 hours, until the experiment was interrupted.

Fig. 9. Kymograph record showing the beneficial action of dog serum after a failure of normal salt solution. *Record I*, normal blood pressure of 112. *Record II*, after exposure of the abdominal viscera for 1 hour; blood pressure 75. *Record III*, after the viscera had been replaced for 10 minutes; blood pressure 65; 156 cc. (20 cc. per kgm.) of normal salt solution were injected (signal A-B). The blood pressure increased to a maximum of 85, but in 15 minutes had decreased to 72. *Record IV*, an equal amount of dog serum was injected (signal C-D). Blood pressure increased to a maximum of 120. *Records V, VI, VII, VIII* and *IX*, were taken at succeeding hours after the injection, with blood pressures respectively of 105, 110, 120, 115 and 105.

Fig. 10. Kymograph record showing the beneficial results of citrated blood after a failure of acacia. *Record I*, normal blood pressure 130. *Record II*, after exposure of the abdominal viscera for 1 hour; blood pressure 80. *Record III*, after the replacing of the viscera for 15 minutes; blood pressure 70; 116 cc. (20 cc. per kgm.) of a 6 per cent acacia solution in 0.9 per cent sodium chlorid solution were injected slowly (signal A-B). The blood pressure increased to a maximum of 90, but soon began to decrease. *Record IV*, taken 30 minutes after the injection was stopped. Blood pressure 60. *Record V*, taken 1 hour after the injection; blood pressure 70. *Record VI*, taken 1½ hours after the injection; blood pressure 74; 116 cc. of citrated blood were injected slowly (signal C-D). The blood pressure increased to a maximum of 110 and then decreased slightly after the injection was stopped. *Record VII*, taken 30 minutes after the injection was stopped; blood pressure 118. *Record VIII*, taken 1½ hours after the injection; blood pressure 118. *Record IX*, taken 2½ hours after the injection; blood pressure 112. *Record X*, taken 4 hours after the injection; blood pressure 98. The animal was used for another experiment.



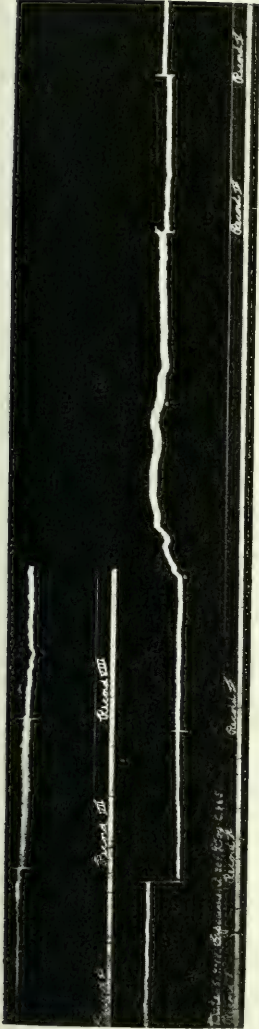


Fig. 8

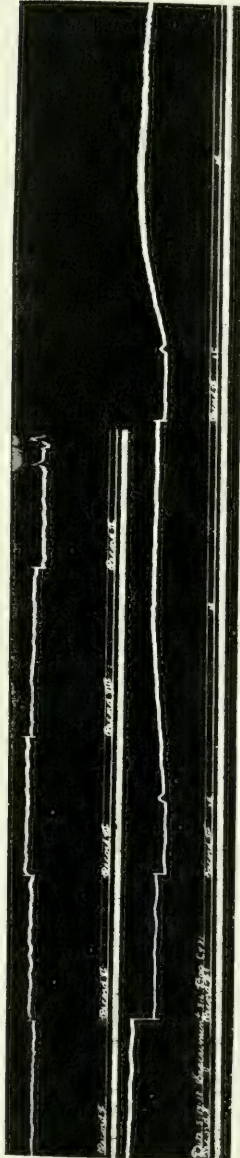


Fig. 9

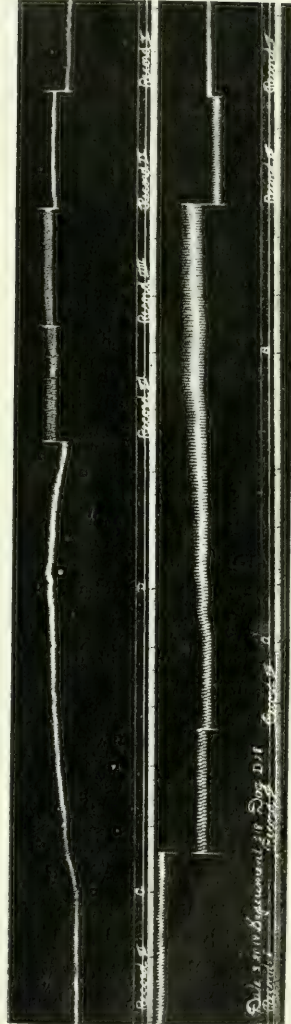


Fig. 10

than one-half its initial value; this is true regardless of the means by which blood pressure is lowered, for example, by hemorrhage, exposure of abdominal viscera, and by obstruction of the venous return, such as partial occlusion of the vena cava. Conclusions should, therefore, not be drawn with regard to a therapeutic procedure when it is tried out in an experiment in which the blood pressure has been decreased below one-half its normal value and because of the variability of the different animals unless, of course, the animal recovers. We believe however, that it might be of some clinical value if a therapeutic procedure could be found which completely or in greater part restores and maintains blood pressure for two hours, with the animal under a constant anesthesia and with a constant artificial temperature condition, after the blood pressure has been decreased from one-third to one-fourth its initial pressure by exposure of the abdominal viscera. These conditions were the standards we used in judging the value of the various methods of treatment.

The treatment of shock may be described under four headings: *a*, general measures; *b*, special measures; *c*, the use of drugs; *d*, attempts to restore fluid volume.

*General measures.* The most important general measure in the treatment of shock is the ancient practice of applying heat. The employment of heat is of value not only because shock is commonly associated with exposure to cold but also because the thermogenetic and thermo-regulatory mechanisms are impaired. It is probably not true that this impairment of the mechanism which keeps the body temperature constant is the primary cause of shock but the artificial maintenance of body temperature during the period of impairment produces beneficial results. It should be noted, however, that this deficiency in regulation applies to heat as well as to cold and that too much heat is harmful.

In most instances the temperature of our animals was kept almost constant by the judicious employment of an electric heating pad. In some experiments the heat was only applied after low blood pressure had been produced and at the same time the other therapeutic measures were instituted. Except that the blood pressure decreased more slowly when the heat was used from the beginning of the experiment no notable difference was observed in the results of the therapeutic procedure.

In order to increase the circulation around the bulbar centers it is usually recommended that the head be placed in slight Trendelenburg

position. Theoretically this should be of value, practically it may be; but in our experiments little effect could be noted.

*Special measures.* The purpose of most of the many special measures which have been devised for treating shock is to increase blood pressure either by decreasing the vascular capacity or by aiding in the return of blood to the heart. Strapping the limb and increasing intra-abdominal pressure should be of benefit, inasmuch as such measures decrease the vascular capacity, but their value is difficult to demonstrate experimentally. Rebreathing has also been recommended; according to Porter, it increases the action of the respiratory pump and thus aids the return of blood to the heart by sucking it into the thorax. The rationale of rebreathing in treating shock from the chemical standpoint is an integral part of Henderson's acapnial theory. The value and limitation of rebreathing in surgery and anesthesia were first carefully studied by Gatch (11). In previous studies on rebreathing in shock I have shown that the process is similar in the normal and in the shocked animal but that no measurable benefit results; this was confirmed by the present series of experiments.

*Drugs.* Drugs are usually employed in the treatment of shock for one of two purposes; first, either as a general stimulant, with particular reference to their action on the circulation and on the central nervous system (strychnin, camphorated oil, alcohol, etc.); and second, as vasomotor constrictors (epinephrin, pituitrin, etc.). Following the theory that shock is due to excessive vasoconstriction, the nitrites have been recommended for the purpose of decreasing vasoconstriction. Morphin is also recommended, mainly for its depressing action on the central nervous system.

Many investigations have been made with regard to the value of strychnin in the treatment of shock. Most investigators are agreed that the drug is of no value, although many surgeons, relying on their clinical experience, still use it in large doses (9). In experimental shock it is impossible to observe any effect of strychnin in doses smaller than those necessary to produce definite convulsive movements. It is questionable whether even these large doses produce a beneficial action; in our experiments it could not be said that any of the so-called stimulants were of value.

The value of the use of vasoconstrictors in the treatment of shock is still an open question. In the first place, although the decreased blood pressure is of great importance in shock, it is not known whether or not its increase by means of vasomotor constrictors is in itself of much



permanent benefit to the organism, and it would seem that they might be of distinct harm by decreasing the fluid supply to the tissues. In the second place, none of the vasoconstrictor drugs produce a very prolonged effect. Epinephrin is the most popular of these drugs to be employed in shock. It easily restores the decreased blood pressure and by continuous injection, the blood pressure can be maintained for a considerable length of time. As soon as the injections are stopped, however, the blood pressure sinks to its former level or usually lower. In our experiments pituitary extract produced a more prolonged action and seemed to be of somewhat greater benefit than epinephrin. Of course, repeated doses of the former cannot be employed as in the case of the latter drug. In general it may be said that experimentally the vasoconstrictor drugs produce little if any permanent benefit in the treatment of surgical shock although they might be employed clinically.

The nitrites produce their characteristic depression of blood pressure when it has been decreased by exposure of the abdominal viscera, but certainly no beneficial result has been observed from their use. Neither is the effect of morphin marked, but since it has been shown that the drug changes the regulations of blood volume, it should be studied more fully (2).

*Attempts to restore fluid volume.* It has been shown that a definite and marked loss of circulating fluid accompanies low blood pressure after the exposure of the abdominal viscera (18). This also seems to be true in other forms of experimental shock (12). In many clinical cases of surgical shock there is a loss of circulating fluid (4), (5), and it seems logical to treat the condition by an attempt to restore the lost fluid to the circulation. A large number of artificial fluids have been devised for this purpose. We have investigated the use of most of these solutions under the standardized conditions mentioned.

We usually injected the fluid to be tested with a burette although in some instances a continuous injection machine was employed. The former method proved the most practical, although it was impossible accurately to control the rate of injection. The effect of the injection depends somewhat on the rate at which the solution enters the vein. In general it seemed that the best results were produced with a rate that was just a little less than the amount which produced cardiac disturbance. Better results were obtained when the temperature of the solution was below 37° rather than above.

In our experiments the use of blood gave far better results than the use of any other substance except blood serum. If blood pressure is



not decreased to less than one-half its initial value after exposure of the abdominal viscera, the intravenous injection of citrated blood in relatively large amounts, 20 cc. per kgm., will practically always restore and maintain it for many hours. As a rule, equally good results have not been secured with any of the artificial solutions. Blood frequently restored blood pressure after other solutions had failed. Homologous blood serum will produce practically the same results (19).

Blood or blood serum show in many ways their superiority over all artificial solutions. They do not raise blood pressure more than some of the other solutions and quite frequently the blood returns more slowly to normal than after the use of some artificial mediums, but, whereas in most instances in which artificial mediums are used blood pressure soon drops to the shock level or below, after the injection of blood or serum, the increase in pressure is usually maintained for many hours. In shock the injection of any solution brings about a return of sensibility requiring higher ether tensions. The degree of sensibility is more marked after the injection of blood or serum than after any one of the solutions.

Physiologic sodium chlorid solution is usually employed to restore lost fluid volume; this is the least valuable of the artificial fluids used if the blood pressure has been lowered in the manner employed by us. Hypertonic saline solutions have been recommended and in some of our experiments they produced a definite beneficial action but the increased blood pressure was never long maintained. None of the saline solutions alone will maintain blood pressure for more than a very short time even when it has been reduced to but slightly below normal by exposure of the abdominal viscera. The saline solutions will usually pass out of the vascular system almost as fast as they are run in.

The use of sodium carbonate and bicarbonate in hemorrhage and shock was experimentally investigated several years ago; their use clinically has been emphasized recently.

Howell (16) seems to have been the first to study the effect of an alkaline salt in shock. He studied the effect of injection of sodium carbonate in a condition of shock produced by different methods. The beneficial results of such injections in the experimental conditions of low blood pressure which he had produced were due, he concluded, chiefly or entirely to a direct action on the heart. Dawson (6) in continuing Howell's study, investigated the effect of the injection of sodium bicarbonate in a condition of low blood pressure produced by hemorrhage. He found that it produced better results than the

sodium chlorid solution, and suggested that the bicarbonate solution be used in those cases of shock accompanied by hemorrhage. Seelig, Tierney and Rodenbaugh (23) obtained marked beneficial results by the injection of sodium carbonate in the condition of experimental shock, and concluded that the results were not due to the bulk of fluid injected, the hypertonicity or alkalinity of the fluid, or to the free carbon dioxid, but to the specific action of the salt on the heart muscle.

Cannon (4), in his study of shock in the front line trenches, found that there is a definite decrease in the alkalinity of the blood in cases of shock. The injection of sodium bicarbonate relieved this and produced very marked benefit. Patients on whom the surgeon refused to operate were tided over the critical period by the injection of either sodium carbonate or sodium bicarbonate which produced a rise in blood pressure and especially an increase in pulse pressure, thus making it possible to operate in a very short time.

In our experiments more lasting benefit was secured by the injection of sodium carbonate or sodium bicarbonate than by normal salt solution. Neither of the alkaline salts, however, completely restored blood pressure, nor was the increase long maintained.

Glucose has been suggested and used in post-operative treatment by several clinicians (3). Erlanger and Woodyatt (8) investigated its action in experimental shock and found it to be of some benefit. In our experiments the injection of such solutions was of definite value although rarely was there a complete restoration of blood pressure, nor was the increase long maintained. Glucose when added to some of the other artificial solutions seemed to enhance their value.

Hogan (15) first recommended gelatine as a medium to restore lost fluid volume. His formula was used in several of our experiments and gave good results in some. In general it is as satisfactory as any of the artificial mediums. Great care should be taken, however, in its preservation, because it deteriorates very readily and may produce untoward results. It was very difficult to modify the gelatine solutions by the addition of other substances, and no modification was found to be as safe or to give better results than Hogan's original formula.

We have used acacia and its various modifications as recommended by Bayliss (1). The addition of acacia to a transfusion solution certainly increases the power of that solution to restore and maintain blood pressure. The results following the use of acacia, however, were quite variable and sometimes disastrous. This variability of

action seemed to depend on both the acacia and the condition of the animal. It is quite possible that the results of our use of acacia have not been so good as those which others report because our acacia was not the same (21). We obtained the best we could, however, and I am quite sure the average surgeon who wishes to use it clinically would obtain no better. The alkaline acacia solution, when properly made by the addition of sodium carbonate or sodium bicarbonate, usually produced a better result than acacia alone, but it is difficult to prepare an alkaline acacia solution and more difficult to sterilize it and, on the whole, it did not seem to be a safe solution to use. Good results were produced by the addition of glucose to acacia (7). The modified acacia solution, which gave the best results in our experiments, consisted of 6 per cent acacia, 10 per cent glucose and 1 per cent sodium sulphate.

Many other methods for restoring fluid volume besides those already mentioned were tried. The rapid injection of 35 per cent solution of cane sugar, as recommended by Guthrie (13), usually fully restored the blood pressure but it was not long maintained. In acute hemorrhage, however, it produces good results. Various strengths of dextrine solutions were used; they restored the blood pressure more satisfactorily than the other artificial solutions but they failed to maintain it. A 1 per cent sodium sulphate solution produced fair results. It is interesting to note that distilled water gives better results in experimental shock with regard to blood pressure than do normal salt solutions. Crude preparations of hemoglobin from dog's blood produced good results and seems to warrant future study.

In summarizing the various methods employed to restore fluid volume it should be emphasized that *a*, in these experiments blood or blood serum produced by far the best results; *b*, the colloidal solutions were the best artificial solutions used; *c*, in general the gelatine solutions produced a more favorable action than the acacia solutions although some of the modifications of the acacia solutions produced as good or a better action than the gelatine; and *d*, care must be exercised in the use of gelatine and acacia because dangerous reactions may be produced with either.

#### SUMMARY

All the more important methods of treating under standard experimental conditions a state that exhibits the clinical signs of surgical shock which is produced by the exposure of the abdominal viscera of a dog, under a constant ether anesthesia, until blood pressure de-

creases to the desired level, were tested. The therapeutic measures were tested after the viscera had been replaced and after determining the curve of the blood pressure.

The treatment of shock is described under four headings:

1. *General measures.* Heat, keeping the head down, etc. The value of the classical use of heat as well as the effect of cold in helping to produce the condition, was corroborated experimentally.

2. *Special measures.* Strapping the limbs, rebreathing, etc. Experimentally, rebreathing was not found to be of importance.

3. *The use of drugs.* Stimulants, vasoconstrictors. None of the drugs usually employed in the treatment of shock were found to be very effective.

4. *The restoration of fluid volume.* The best results in the treatment of experimental shock were obtained by the injection of fluid media. The data of the experiments justify the conclusion that none of the artificial solutions give such good results as the use of blood. The so-called colloidal solutions and their various modifications give better results than normal salt solution, but their potency is certainly not equal to blood or blood serum and occasionally they might be harmful.

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# EXPERIMENTAL STUDIES ON THE REGULATION OF BODY TEMPERATURE

## III. THE EFFECT OF INCREASED INTRACRANIAL PRESSURE ON BODY TEMPERATURE

(Preliminary Communication)

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In a previous paper I (1) reported a series of "heat puncture" experiments on rabbits, the results of which indicated that in certain cases hyperthermia followed puncture of the brain, but since there was no correlation between the location of the lesion and the occurrence of the hyperthermia, the rise in temperature did not depend upon injury to the corpus striatum or other special "center." The existence of special "centers" in the brain for the regulation of body temperature was, therefore, not confirmed.

The hyperthermia which occurred in 22 per cent of the cases, however, remained unexplained, as also did the fatal symptoms accompanying a number of these and other cases of puncture; the symptoms noted in such cases suggested the possibility of a condition of increased intracranial pressure due perhaps to hemorrhage in the cranial vessels. This possibility was tested out by a series of experiments in which the intracranial pressure in rabbits was increased artificially. The symptoms produced by greatly increasing the pressure, 250 mm. or more of water, were identical with those observed in fatal puncture cases; increase in rate of respiration, slowing of heart beat, vasoconstriction, pupilo-dilatation, rise in body temperature followed by a fall before death. A moderate increase (150 to 200 mm. of water) produced less marked effects, the most apparent being an increase in the rate of respiration and vasoconstriction with a subsequent rise in body temperature. Practically every case with a definite increase in the pressure in the cranial cavity showed a rise in temperature sufficiently high to

be considered experimentally produced. The findings accord with the well-known symptoms in clinical cases of traumatic brain lesions.

The explanation of this influence of increased intracranial pressure on body temperature and also the fatal symptoms of higher pressure would appear to be that the pressure acts as a stimulus to the principal bulbar centers causing an increased rate of respiration by stimulation of the respiratory center, decrease in heart rate by stimulation of the cardio-inhibitory center, vasoconstriction by stimulation of the vasomotor center, and dilatation of the pupils by stimulation of the cervical sympathetic. In case of greatly increased and prolonged pressure these centers finally become paralyzed, their functioning ceases and death follows. Dixon and Halliburton (2) offer a similar explanation of the pressure symptoms observed by them in their experiments with dogs. On the other hand, with a moderate increase in intracranial pressure there is less evidence of bulbar stimulation, the most apparent excitation being that of the vasomotor center. The vasoconstriction resulting from this stimulation causes more heat to be retained in the body and consequently a rise in the body temperature. In fatal cases the body temperature falls again just preceding death probably because of the paralysis of the vasomotor center.

These results support the view that temperature regulation is dependent upon physico-chemical factors without the intervention of hypothetical "heat centers."

The rise in body temperature and other symptoms attending increased intracranial pressure correspond so closely to those of "heat puncture" in which there is generally sufficient brain lesion to cause an increase in pressure in the brain cavity, and those of clinical brain lesions, that it seems possible to apply the same explanation to each of these cases. I think the rise in temperature which is reported by advocates of the "heat center" theory as due to "heat puncture" can be explained in a like manner, as can also the rise obtained in 22 per cent of my punctures and the fatal symptoms in a number of these and other cases.

The detailed results of this and further investigations will be published in a later paper.

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## STUDIES IN SECONDARY TRAUMATIC SHOCK

### V. RESTORATION OF THE PLASMA VOLUME AND OF THE ALKALI RESERVE

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The method of estimating the blood volume employed in the preceding paper (1) brought to light the fact that the intravenous injection of a concentrated solution of gum acacia tends to prevent the concentration of the blood which otherwise practically invariably develops while shock is being induced. This observation suggested the present set of experiments which had as their first object a more detailed inquiry into the mechanism of this action of gum acacia, and ways of facilitating it.

#### SERIES I. OBSERVATIONS ON NORMAL ANIMALS

*Procedure.* Three experiments were performed on each of four normal dogs. The animals were anesthetized with morphine and ether. The first experiment on each dog consisted in the administration of a hypertonic crystalloid (18 per cent glucose, usually 5 cc. per kilo of body weight). After the animal had recovered from the effects of this injection, usually on the next day, a hypertonic colloid (5 to 6 cc. of a 30 or 25 per cent solution of gum acacia, respectively) was given. An interval of 3 days was then allowed to elapse. This was regarded as sufficiently long to permit of the disappearance of the gum acacia. Then in three of the instances the animal was given, first, the acacia and, immediately after, the glucose, both in the same concentration and amount as had been previously employed. To the fourth animal was given a 7 per cent solution of gum acacia. All injections were made as rapidly as possible. The hemoglobin percentage, determined as described in the preceding paper (1), served to indicate the effects these injections had upon the blood volume. The injections were made into the femoral vein and the samples of blood were taken



from some large artery. The arterial pressure was not followed in this series of experiments.

*Results.* When glucose is injected, the well-known fact is confirmed that the blood comes into osmotic equilibrium with the tissues within the first minute or two. The maximum theoretical dilution produced by the dose of glucose given may be calculated thus:—A 5.52 per cent solution of glucose is isotonic with blood

$$\left( \frac{6.88 \text{ (osmotic pressure of blood in atmospheres)} \times 18}{22.4 \text{ (osmotic pressure of 18 per cent glucose)}} = 5.52 \right);$$

the 0.9 gram of glucose contained in each 5 cc. of the solution, the dose per kilo of animal, would therefore suffice to make  $\frac{0.9}{0.0552}$ , or 16.3 cc. of an isotonic solution. If we take 92 cc. as the quantity of blood in

TABLE 1

| (1)<br><br>EXPERIMENT<br>NUMBER | GLUCOSE                  |   |   | ACACIA                   |   |   | ACACIA AND GLUCOSE       |   |
|---------------------------------|--------------------------|---|---|--------------------------|---|---|--------------------------|---|
|                                 | (2)<br><br>Dose per kilo | (3)<br><br>Theoretical in-<br>crease in vol-<br>ume | (4)<br><br>Observed maxi-<br>mum volume<br>increase | (5)<br><br>Dose per kilo | (6)<br><br>Theoretical in-<br>crease in vol-<br>ume | (7)<br><br>Observed maxi-<br>mum volume<br>increase | (8)<br><br>Dose per kilo | (9)<br><br>Observed maxi-<br>mum volume<br>increase |
|                                 |                          | per cent  | per cent  |                          | per cent  | per cent  |                          | per cent  |
| 1                               | 3 cc. 18%                | 10.6  | 7.0   | 5 cc. 30%                | 31.2  | 19.0  | { 5 cc. 30%<br>3 cc. 18% | 24.0  |
| 2                               | 5 cc. 18%                | 17.7  | 6.0*  | 6 cc. 25%                | 31.2  | 10.0  | { 6 cc. 25%<br>5 cc. 18% | 18.0  |
| 3                               | 5 cc. 18%                | 17.7  | 12.0  | 6 cc. 25%                | 31.2  | 11.0  | { 6 cc. 25%<br>5 cc. 18% | 35.0  |
| 4                               | 5 cc. 18%                | 17.7  | 10.0  | 5 cc. 30%                | 31.2  | 12.0  | 18 cc. 7%                | 22.0  |

\* Solution administered too slowly.

each kilo of animal the addition of 16.3 cc. per kilo will make a blood volume of 108.3 cc.; the blood volume is increased 17.7 per cent. Of the water required to effect this increase 5 cc. are furnished by the injection; the remaining 11.3 cc. must be taken from the tissues.

The results obtained are given in table 1, and two of the four sets of observations are plotted in figures 1 and 2. The table shows that in

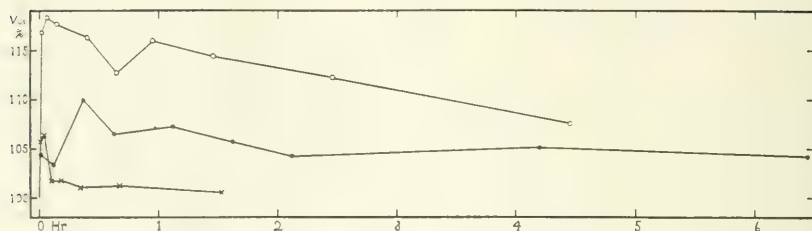


Fig. 1. Experiment 2, table 1. Blood volume, in per cent of the original, plotted against the time. Zero hour marks the completion of the injection of 5 cc. of 18 per cent glucose (—x—x—) on the 1st day, 6 cc. of 25 per cent gum acacia (—●—●—) on the 2nd day, and 6 cc. of 25 per cent gum followed immediately by 5 cc. of 18 per cent glucose (—○—○—) on the 4th day.

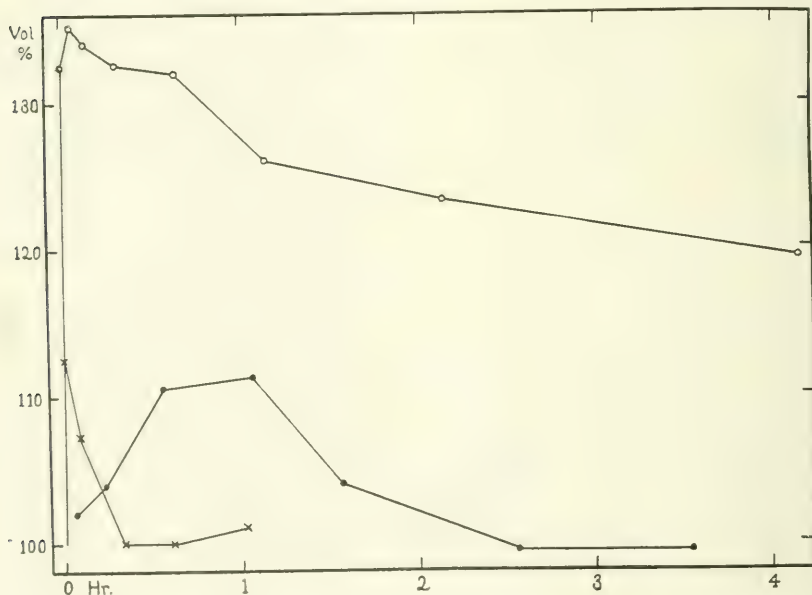


Fig. 2. Experiment 3, table 1. Blood volume in per cent of the original plotted against the time. Zero hour marks the completion of the injection of 5 cc. of 18 per cent glucose (—x—x—) on the 1st day, 6 cc. of 25 per cent gum acacia (—●—●—) on the 2nd day, and 6 cc. of 25 per cent gum followed immediately by 5 cc. of 18 per cent glucose (—○—○—) some days later.

each instance the observed maximum dilution after the injection of glucose (average = 8.7 per cent) falls far short of the theoretical maximum (17.7 per cent). This difference, presumably, is merely the result of the rapid loss of glucose from the circulation; it may be disappearing while the injection is proceeding, and also during the short interval elapsing between the termination of the injection and the taking of the first sample of blood. The largest discrepancy is seen in experiment 2, and in this instance it was recorded that the injection proceeded too slowly.

The maximum dilution was attained within  $\frac{1}{2}$  to 2 minutes. The blood then began to concentrate, rapidly at first, and then more slowly, and became normal within 5 to 45 minutes.

The results *following the injection of the acacia solution* differed quite decidedly from those obtained with the glucose. We present first our method of calculating the anticipated dilution of the blood as based upon the osmotic pressure of the acacia solution. According to data presented in the preceding paper (1) we can consider the osmotic pressures of 7 per cent acacia and blood serum 22 mm. and 16.4 mm. of mercury, respectively. On this basis 5 cc. of 30 per cent acacia would have to expand to 28.7 cc. to have the same osmotic pressure as the serum colloids. This would entail a 31.2 per cent expansion of the blood volume (normal 92 cc. per kilo).

The maximum dilutions observed experimentally (19, 10, 11 and 12 per cent) were in every instance much less than the theoretical 31.2 per cent. The dilution of 19 per cent was obtained in an experiment in which acacia was injected as soon as the dilution resulting from the glucose had disappeared; in the other experiments the acacia was injected one to three days after the sugar. This maximum dilution in every instance was attained very much more slowly (in 25 to 50 minutes) than when the glucose was injected (in  $\frac{1}{2}$  to 2 minutes). Of greater interest, however, is the slow return of the blood concentration toward the normal. In the four experiments the return to normal required more than 3 hours, more than 6 hours,  $2\frac{1}{2}$  hours and 3 hours respectively.

It is obvious that the colloid attracts water very slowly compared with the crystalloid glucose; therefore, at no time during the period of observation does the serum regain its normal osmotic properties. In spite of the increased colloid content of the plasma the maximum volume reached is not maintained. The plasma volume is reduced toward normal in spite of its increased colloidal osmotic pressure. This is

most probably to be attributed to filtration resulting from the increase in capillary pressure that would arise from the plethora and the increased viscosity. As acacia can maintain for some time a volume greater than normal it is to be expected that it would be even more effective when injected into an animal whose blood volume had been previously depleted, the volume-compensating mechanism not being then called into play. This is an example of the well-known pharmacological principle that it is easier to change a function toward normal than away from normal.

It follows from these observations that neither the concentrated solution of gum acacia nor the concentrated solution of glucose is perfectly satisfactory as a means of expanding the blood volume; the former attracts water slowly, though it holds it in the circulation for relatively long periods of time, whereas the latter attracts water with great rapidity, but the increased blood volume disappears with corresponding rapidity. To maintain an increased volume by the injection of glucose, the injection must be continuous.

It was conceived, therefore, that the shortcomings of each of these methods of expanding the blood volume might be corrected by combining the two: that if concentrated acacia were injected it would hold the water which a subsequent injection of concentrated glucose brought it. Experiment proved this to be true.

*When glucose is injected after acacia* we might expect, if the acacia holds all the water that the sugar can bring in, that the maximum theoretical blood volume increases of 10.6, 17.7 and 17.7 per cent respectively in the first three experiments would be added to the blood volumes resulting from the acacia injection. If we add the former percentages to the percentage increases obtained experimentally in the acacia control series (columns 3 and 7, table 1) we have theoretically possible expansions of 29.6, 27.7 and 28.7 per cent, that is, volumes within the theoretical holding power of the injected acacia. The corresponding expansions found were respectively 24.18 and 35 per cent (column 9). Explanation of the variations from theory would demand a longer and more carefully controlled series. The data, at any rate, clearly support the original supposition, that glucose would bring in fluid which would be held by the acacia. The maximum dilution was reached very quickly; indeed, in two of the instances it was attained within 4 minutes or practically as quickly as when glucose was injected alone. In the other case the maximum was attained with the reading made 16 minutes after the injection, but 8 minutes had elapsed between this and the preceding reading.



After attaining the maximum, the blood volume at once begins to decline. In the two instances in which the maximum was quickly attained (experiments 2 and 3) this decline was slowly progressive as long as it was followed. At the time of the last readings made, the blood volume has always been higher following the injection of the two solutions than in the case of the injection of acacia alone. This is probably due in part to the greater initial increase in volume when the gum and the crystalloid are injected together, although it has been our experience in several instances that the increase in volume resulting from a rapid injection of very hypertonic colloid may be rapidly disposed of. The reason for this we have not analyzed.

The slow return of the blood volume to normal is seen also after the injection of more dilute (7 per cent) acacia. In the third part of experiment 4, 18 cc. per kilo of such a solution were injected, a volume sufficient to increase the blood volume (normal 92 cc. per kilo) 19.6 per cent. The actual dilution observed was about 21 per cent directly after the injection. The blood volume began to decline at once, but so slowly that 5 hours later it was still 111 per cent of the original. The 7 per cent acacia was chosen in this experiment on the assumption that we were working with an isotonic colloid. According to our present calculations, however, even the 7 per cent would be appreciably hypertonic.

#### SERIES II. OBSERVATIONS ON ANIMALS IN SHOCK

##### *1. The effect of injection of solutions of colloids and crystalloids on the circulation*

The main conclusion to be drawn from the experiments reported in part 1 of this paper is that in normal animals a hypertonic colloid will hold in the circulation not only the water that it itself slowly attracts, but also the water brought to it rapidly by a hypertonic crystalloid, so that the combined injection of the two results in a rapid and well-sustained expansion of the blood volume. The colloid employed in those experiments was gum acacia; the crystalloid, glucose. In view of the acidosis demonstrated to be present in the types of shock with which we were working it was deemed advisable in the tests carried out on animals in shock to employ as the crystalloid either  $\text{Na}_2\text{CO}_3$  or  $\text{NaHCO}_3$ , instead of glucose. It may be assumed that the principles involved, as regards changes in the blood volume, are not altered by this change in the crystalloid employed.

*Methods.* The animals were first traumatized by holding the arterial pressure down to 40 mm. Hg. for a period of about 2 to  $2\frac{1}{4}$  hours by partly occluding the inferior vena cava (2), (3). This damage in our experience in time usually brings on a shock-like failure of the circulation. The blood volume was followed, as previously, by estimating the hemoglobin. In addition, the pressure in the carotid artery was followed by a method that entailed practically no loss of blood or mixture of the anticoagulant salt with the blood in the animal. In a few experiments the  $\text{CO}_2$  capacity of the blood was followed by the Van Slyke method.

In most of the experiments the solution injected was 25 per cent sodium acacia in 4 or 5 per cent  $\text{Na}_2\text{CO}_3$  or  $\text{NaHCO}_3$ , the amount, 4 or 5 cc. of the solution per kilo of body weight, though many other combinations, including the first Bayliss solution (4), were used. As a 1.37 per cent solution of  $\text{Na}_2\text{CO}_3$  is isosmotic to blood, each cubic centimeter of a 5 per cent solution injected would have to expand to 3.65 cc. before becoming isosmotic to plasma. Our 5 per cent solution of  $\text{NaHCO}_3$  lowered the freezing point of water  $1.879^\circ\text{C}$ . One cubic centimeter of this would, therefore, expand to about 3.35 cc. Five per cent solutions of these substances are, therefore, comparable with respect to their osmotic pressures to the 18 per cent glucose in the preceding experiments. Owing to the inadvisability of rapidly injecting concentrated acacia solutions into animals in shock, the injection in these experiments was prolonged usually to 20 or more minutes.

*Blood volume.* The changes in the blood volume that occurred are collected in table 2. In every case the increase in volume after the injection of 25 per cent gum in combination with 4 or 5 per cent carbonate is at least as great as that calculated to be necessary for all the crystalloid injected to become isosmotic. And in no instance is the increase in blood volume, determined shortly after the injection, any greater than the volume of water that the injected gum acacia is theoretically capable of holding. But in experiments 22, 25 and 30 the blood volume continues to increase so that the ultimate dilution is greater than the theoretical holding power of the acacia, though within the limit of error of the methods, in the case of experiment 30. The explanation of this result is not obvious. There is the possibility that the improvement in the circulation permits the organism to participate in increasing the blood volume.

A glance at the table will show that other solutions of gum acacia, such as simple 7 per cent gum acacia and 6 per cent gum acacia in

TABLE 2

| EXPERIMENT NUMBER  | TREATMENT  | BLOOD VOLUME CHANGES FROM INJECTION |                  |                    |                      |           |         |                      |         |                   |                                | RESULT |  |
|--------------------|--|-------------------------------------|------------------|--------------------|----------------------|-----------|---------|----------------------|---------|-------------------|--------------------------------|--------|--|
|                    |  | SHOCK VOLUME                        | Maximum          |                    |                      |           |         | Last observation     |         |                   | ARTERIAL PRESSURE              |        |  |
|                    |  |                                     | Within 3 minutes | Volume             | Time after injection | In-crease | Volume  | Time after injection | Initial | Before treat-ment | Imme- diately after treat-ment |        |  |
|                    |  |                                     |                  |                    |                      |           |         |                      |         |                   |                                |        |  |
| per cent of normal | per cent of normal   | per cent of normal                  | minutes          | per cent of normal | per cent of normal   | minutes   | mm. Hg. | mm. Hg.              | mm. Hg. |                   |                                |        |  |
| 22                 | 25% acacia in 5% $\text{Na}_2\text{CO}_3$ , 5 cc. per kilo   | 90.2                                | 115.8            | 131.0              | 67                   | 45.0      | 125.2   | 120                  | 105     | 130               | Died                           |        |  |
| 25                 | 25% acacia in 5% $\text{Na}_2\text{CO}_3$ , 5 cc. per kilo   | 84.0?                               | 115.0            | 127.2              | 78                   | 51.4?     | 127.2   | 78                   | 140     | 45                | 70                             | Died   |  |
| 24                 | 25% acacia in 5% $\text{Na}_2\text{CO}_3$ , 4 cc. per kilo   | 87.6                                | 104.2            | 104.2              | 2                    | 19.0      | 98.2    | 121                  | 120     | 95                | 130                            | Lived  |  |
| 30                 | 25% acacia in 5% $\text{Na}_2\text{CO}_3$ , 4 cc. per kilo   | 85.6                                | 109.0            | 112.6              | 100                  | 31.6      | 112.6   | 100                  | 130     | 80                | 84                             | Died   |  |
| 34                 | 25% acacia in 4% $\text{NaHCO}_3$ , 4 cc. per kilo           | 80.0                                | 94.0             | 94.0               |                      | 17.4      | 94.0    | 62                   | 120     | 95                | 107                            | Died   |  |
| 43                 | 25% acacia in 4% $\text{NaHCO}_3$ , 5 cc. per kilo           | 75.9                                | 91.9             | 91.9               |                      | 21.0      | 91.9    | 6                    | 100     | 115               |                                | Lived  |  |
| 41                 | 25% acacia in 4% $\text{NaHCO}_3$ , 4 cc. per kilo           | 80.0                                | 100.4            |                    |                      |           | 100.4   | 24                   | 105     | 92                | 100                            | Died   |  |
| 18a                | 7% acacia in 2.4% $\text{Na}_2\text{CO}_3$ , 24 cc. per kilo | 88.8                                | 122.4            | 122.4              | 3                    | 37.8      | 111.0   | 128                  | 105     | 90                | 110                            | Died   |  |
| 28                 | 6% acacia in 2% $\text{NaHCO}_3$ , 12 cc. per kilo           | 85.7                                | 111.7            | 114.3              | 13                   | 33.4      | 114.0   | 13                   | 120     | 58                | 68                             | Died   |  |
| 18                 | 6% acacia in 2% $\text{NaHCO}_3$ , 18 cc. per kilo           | 86.8                                | 108.2            | 113.2              | 109.2                | 30.4      |         |                      | 110     | 98                | 115                            | Died   |  |
| 17                 | 7% acacia, 18 cc. per kilo                                   | 81.6                                | 94.8             | 95.8               | 32                   | 17        | 92.2    | 80                   | 180     | 187               | 80-120                         | Died   |  |

2 per cent  $\text{NaHCO}_3$  (4), when osmotic properties as well as volume of fluid injected are taken into account, are quite as efficacious in restoring and maintaining the blood volume as are the stronger solutions. While there is often some recession from the maximum dilution this is never large, and in five of seven animals in which the concentration was followed for over 1 hour after the injection, the volume at the last reading is as large as that obtaining within the first 3 minutes after the injection.

When these experiments were performed we believed that 7 per cent acacia was isosmotic with the colloids of the blood and that its effect on the blood volume was merely additive. On the basis of data presented in the preceding paper (1), we now consider the colloidal osmotic pressure of serum to be 16.4 mm. of mercury and of 7 per cent acacia 22 mm. We have recently dialyzed a 7 per cent solution of acacia in the same Locke's solution used in making the above determinations, against serum in a Moore and Roaf osmometer, arrangement being made for intermittent renewal of the serum in the lower chamber. The pressure developed was 6.6 mm. of mercury. The pressure on the acacia was released after the first determination and the experiment then continued with the salts in the acacia solution modified by the interchange of salts with the serum during the first determination. The pressure developed in the second determination was again 6.6 mm. This pressure is to be compared with the difference of 5.6 mm. between the separate direct determinations.

After the injection of 18 cc. per kilo of 7 per cent acacia the osmotic pressure of the plasma colloids is calculated to be 8.5 per cent above normal. After the injection of 5 cc. per kilo of a solution containing 18 per cent glucose and 25 per cent acacia the colloids would be 10.7 per cent hypertonic after enough fluid had been brought in from the tissues to render the crystalloid isotonic, assuming that all the glucose stayed in the vessels till diluted. It therefore follows that after the injection either of the 7 per cent acacia solution or of the very hypertonic gum and glucose solutions in the above quantities the blood plasma is left with a colloidal osmotic pressure somewhat above normal.

In the solution containing 6 per cent acacia and 2 per cent  $\text{NaHCO}_3$  both the colloid and crystalloid are slightly hypertonic, even after making allowance for the loss of carbonate in the precipitation of  $\text{CaCO}_3$ . Table 2 shows that the blood dilution resulting from the injection of the 6 per cent gum acacia solution in 2 per cent  $\text{NaHCO}_3$  was greater



than could be accounted for simply by the addition of the volume of water actually injected.

*Blood pressure.* Practically invariably the arterial pressure is raised by the injection of any of these solutions containing gum and crystalloid (see table 2) even when the injection of the crystalloid alone is without effect (see *a*, fig. 6). The effect of the injection upon the blood pressure, however, seems to be merely an incident in the course taken by the pressure as determined by the reaction of the animal to the clamping of the cava. If at the time the gum solution is injected the pressure is rising, the injection facilitates the rise somewhat; but if the pressure is falling, the solution while usually causing the pressure to rise for a time, fails to maintain the pressure. Sooner or later the pressure begins to fall again, and the animal invariably dies. This fall in pressure may occur even while the blood volume apparently is increasing.

## 2. Alkali reserve in experimental shock

Before presenting the effect on the alkali reserve of the acacia-carbonate mixtures, the effect of the shock-producing procedures which we have employed, upon the carbonate content of the plasma should be recorded. This study was made because at the time these experiments were performed considerable importance was being attached to the reduction in reserve alkali which had been found to obtain in clinical shock (5).

In a number of animals we have followed with the Van Slyke apparatus the changes in the  $\text{CO}_2$  capacity of the plasma of arterial blood drawn without loss of  $\text{CO}_2$ . This is regarded by Van Slyke and Cullen (6) as the ideal method of estimating the alkali reserve. Most of the determinations were made in duplicate.

The data are collected in figure 3 in which the volume per cent of  $\text{CO}_2$  is plotted against the mean arterial pressure. The first blood sample in each case was drawn after anesthetizing the animal with morphine followed by ether and after making all of the operations preparatory to starting the procedures by which shock-like failure of the circulation was to be induced. It is seen that these readings ranged between 50.5 and 26.1, and usually were below 40.0.

In order to avoid any complications that might arise as a result of the withdrawal of blood, it was necessary to reduce the number of readings of the  $\text{CO}_2$  capacity to a minimum. As a rule, therefore, only one other reading was made and that at a time when we felt as-

sured that a shock-like failure of the circulation had become established. The readings made at that time, with but one exception (no. 2), show a markedly reduced  $\text{CO}_2$  capacity. And, with but this one exception, the inclination of the line joining the initial with the shock reading in almost every instance is remarkably constant; these lines are all nearly parallel to each other.

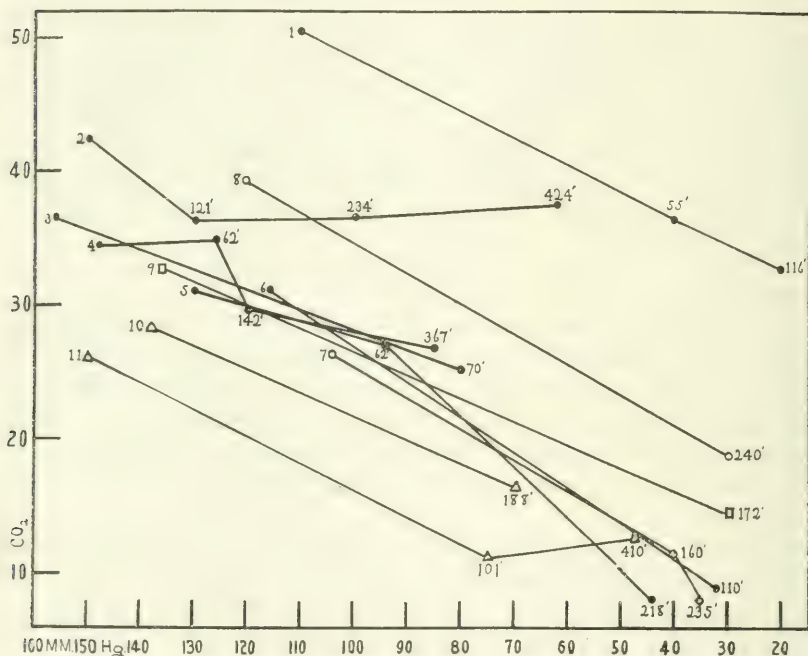


Fig. 3. Showing the relation of  $\text{CO}_2$  capacity of arterial blood (ordinates) to arterial pressure (abscissae). The first reading in each case is made before starting to induce shock; the subsequent readings later by the time (in minutes) indicated in each case. Shock by intestinal exposure, —●—; shock by caval occlusion, —○—; shock by aortic occlusion, —□—; shock by adrenalin injection —△—.

On the other hand the gradient of the curves or of the parts of the curves joining readings made before the fall in pressure in general had exceeded 50 per cent of the original value is not quite so steep. This possibly may mean that the tissue alterations that lead to the reduction in alkali reserve do not progress rapidly until the condition that brings them about has reached a relatively advanced stage of develop-

ment. This inference is borne out in the main by the curves of the relation obtaining between the  $\text{CO}_2$  capacity and the volume flow of blood published by Gesell (7). His curves as a rule show that the flow of blood through the salivary gland may be below 50 per cent of the original for some time before the  $\text{CO}_2$  capacity begins to fall. It would seem, therefore, that the reduced  $\text{CO}_2$  capacity is the result and not the cause of the fall in arterial pressure.

The gradient of the line joining the initial with the final readings as may be seen in figure 3 is largely independent of the time. It can also

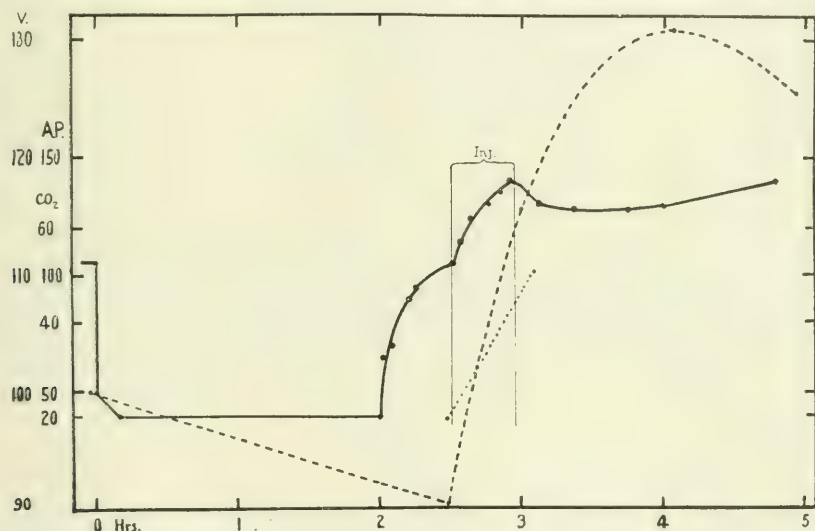


Fig. 4. Experiment 22. Arterial pressure, —●—●—; blood volume, --●--●--, per cent of normal, determined by the reciprocal of the Hb. content;  $\text{CO}_2$  capacity, ..●...●.. The figure shows the acceleration of the rise of pressure, that follows release of the cava, by the injection of 5 cc. per kilo of a solution consisting of 25 per cent sodium acacia in 5 per cent  $\text{Na}_2\text{CO}_3$ , and the subsequent maintenance of a good pressure. It shows, also, a tremendous increase in the blood volume resulting from the injection. The blood volume is still high at the close of the experiment, more than 2 hours after completing the injection. It also shows the rise in the  $\text{CO}_2$  capacity. This animal died before twenty-three hours had elapsed.

be seen that though the lines tend to parallel each other they may be on widely separated levels, so much so that the final readings in a case of advanced shock may be within, or almost within, the range of the normal readings of other cases. While there is no clear direct relationship

between the  $\text{CO}_2$  capacity and the arterial pressure, it nevertheless is obvious that a reduction in the carbonate content of the plasma from arterial blood is a constant accompaniment of shock as we have induced it.

*The alkali reserve after injections of solutions containing acacia and carbonate.* The carbonate content of the arterial blood was followed in two of the cases that received 5 per cent  $\text{Na}_2\text{CO}_3$  with the acacia.

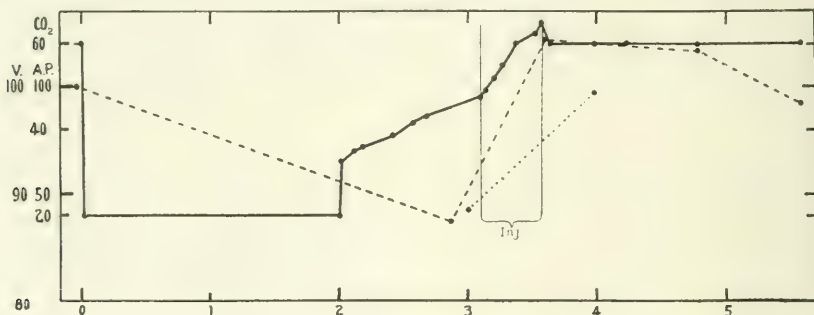


Fig. 5. Experiment 24. Four cubic centimeters per kilo of 25 per cent acacia in 5 per cent  $\text{Na}_2\text{CO}_3$  were injected. Essentially the same results were obtained in this case as in experiment 22. This animal recovered.

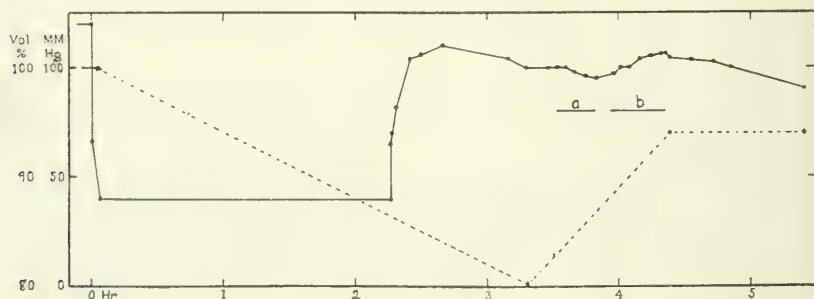


Fig. 6. Experiment 34. In this case the pressure began to fall when something less than 54 minutes had elapsed after removing the clamp from the cava. At a time when the pressure was stationary at 100 mm. of Hg. the animal (at *a*) was given 4 cc. per kilo of a 4 per cent solution of  $\text{NaHCO}_3$ . The pressure fell somewhat while this was being given. A solution consisting of 25 per cent sodium acacia in 4 per cent  $\text{NaHCO}_3$ , 4 cc. per kilo, was then given (at *b*). Under the influence of this injection the pressure rose and the blood volume increased, but neither was quite restored to normal. Almost immediately after terminating the injection the blood pressure began to fall again despite any measureable decrease in blood volume. This animal died.



One received 5 cc. per kilo of body weight, the other 4 cc. In the first case (fig. 4) the volume of combined  $\text{CO}_2$  was raised from the shock level of 19.8 per cent to 50.6 per cent; and in the second case (fig. 5) from 20.6 per cent to 48.6 per cent. The values attained in each case, therefore, were quite normal for dogs under ordinary conditions of experimentation.

*The ultimate results of the injection of a solution containing hypertonic acacia and carbonate.* A general discussion of the effect of the injections on the blood pressure, the blood volume and the alkali reserve has been given. There remains to be considered the fate of the individual animals. In figures 4, 5 and 6 typical experiments are graphically presented. It is obvious from these and similar experiments (see table 2) that an intravenous injection which raises the blood pressure, and maintains the rise for the period of the experiment, which restores the blood volume and the alkali reserve in an animal that has been damaged to an extent that in time usually results in a shock-like failure of the circulation, may not prevent a fatal issue. One cannot help but conclude, therefore, that these alterations in the state of the animal do not constitute the primary cause of death from shock; in other words, they are merely symptoms of some more fundamental process, which determines the condition of the animal but to which the former may be contributory.

#### SUMMARY

1. When glucose in 18 per cent solution is injected into the circulation of a normal animal the blood comes into osmotic equilibrium with the tissues within the first minute or two, the average maximum dilution amounts to but half of the theoretical maximum and the blood regains its normal concentration within 5 to 45 minutes.

2. When gum acacia in a concentrated solution is injected the average maximum dilution of 41.7 per cent of the theoretical maximum is attained within 25 to 50 minutes; the decline of the blood volume to normal requires  $2\frac{1}{2}$  to 6 or more hours.

3. When the concentrated acacia is immediately followed by the glucose the maximum dilution is quickly attained and is much greater than that resulting from the injection of either of the two substances alone. The dilution is well maintained.

4. Comparable results are obtained in animals in shock when a strong solution of gum acacia is followed by a solution of  $\text{Na}_2\text{CO}_3$  that is isosmotic to 18 per cent glucose.

5. With such a combination of solutions given in appropriate amounts the blood volume, the blood pressure and the reserve alkali of animals in shock often can be brought to normal and held there for the usual duration of an experiment. Yet such animals, as well as shocked animals treated with other combinations of gum acacia and carbonate or bicarbonate, often died within 24 hours.

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## STUDIES IN SECONDARY TRAUMATIC SHOCK

### VI. STATISTICAL STUDY OF THE TREATMENT OF MEASURED TRAUMA WITH SOLUTIONS OF GUM ACACIA AND CRYSTALLOIDS

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#### INTRODUCTION

With the growth of our personal experience with experimental shock we came more and more to feel that a solution consisting of hypertonic gum acacia and of a hypertonic crystalloid would prove helpful in counteracting the disturbances which seemed to be responsible for the failure of the circulation that constitutes the main feature of that state.

The growth of this idea may be briefly outlined. Our first experiments had as their object a study of the hemodynamics of shock. They convinced us that a giving way of the vasomotor center or of the heart is to be regarded, speaking broadly, only as a relatively late consequence of a low arterial pressure, and not as its cause. In the early stages of shock as induced by exposure of the intestines (1), by partial occlusion of the aorta or of the vena cava (2), or by the administration of adrenalin (3), the only constant circulatory fault seemed to be a reduction in blood volume, both actual and effective (4). We found in all types of shock, among other less constant lesions, marked dilatation of the capillaries and venules of the villi of the intestines.

Since our methods of inducing shock all seemed to depend upon the effects of slowing the blood stream, since furthermore the same vascular changes can be seen to develop during partial occlusion of an artery (5), and since the shock that follows the administration of adrenalin seems to be referable to the constriction of the arterioles it produces by local action, we ventured to suggest (6) that in man the

<sup>1</sup> With the assistance in some of the experiments of Paul C. Hodges.

prime factor, also, might be a strong vasoconstriction compensating hemorrhage and wound weeping, and aggravated by exposure and pain. The changes thus produced in the capillaries and venules by the slowing of the circulation would account for the concentration of the blood found in experimental shock by attributing it to transudation of plasma and they account for the reduction in effective blood volume through stagnation in the dilated vessels.

Although the disappearance of plasma from the circulating blood is of constant occurrence in shock experimentally produced, there is evidence that while plasma is disappearing, fluids are being added to the blood (4). The latter process may be regarded as an effort on the part of the organism to combat the diminution in blood volume. Evidence has been obtained indicating that the ability of the organism to thus make good the loss in blood volume decreases as shock develops.

It was while we were studying the blood volume in shock that we happened upon the observation that the concentration of the blood that develops during shock induction does not occur, or at least is not so marked, when shock is induced after the administration of a dose of hypertonic gum acacia. This result we were able to show is due in part, at least, to the osmotic pressure the hypertonic gum exerts (7). It was found, however, that hypertonic gum solutions attract tissue fluids into the circulation very slowly. Hypertonic crystalloids injected intravenously, as is well known, attract water quickly. When the two, the hypertonic gum and the hypertonic crystalloid, are injected to all intents and purposes, simultaneously, the gum holds the water the crystalloid quickly brings into the circulation. Presumably, the blood volume is thus increased by a process that resembles the one the organism itself employs in combatting a reduction in blood volume. Inasmuch as the reserve alkali was found to be reduced in the types of shock we were studying (7), it was felt that sodium bicarbonate or sodium carbonate might be made to act at one and the same time as the hypertonic crystalloid and as a supply of base. We therefore studied the action on animals in shock of hypertonic gum acacia and hypertonic bicarbonate, as well as other combinations of gum and crystalloid and found (7) that while all equivalent combinations acted on the arterial pressure, the blood volume and the alkali reserve approximately alike and about as had been anticipated, many of the animals, nevertheless, died.

It thus became obvious that in order to ascertain whether these or other similar solutions that have been proposed or may be proposed



for the intravenous treatment of shock are of value, it would be necessary to study their action on a standard condition, one whose course, if left to itself, could be predicted with a reasonable degree of certainty and to use as the criterion of their efficacy not their effect on the blood pressure, or on the blood volume, or on the alkali reserve, but actual recovery from the state of shock. In the present state of uncertainty of our knowledge with regard to the nature of shock, no other criterion can be convincing. It was felt that the conditions for such a study would be supplied best by exposing an animal to a form of trauma which in time brings on a shock-like failure of the circulation, and to so grade that injury that it would just cause death if things were allowed to take their course. To state this conception in another way, the interpretation of therapeutic tests would be entirely free of ambiguity if they could be made on animals on which a minimal fatal amount of injury had been inflicted, and if recovery from the immediate effects of the damage were taken as the criterion of efficacy.

Of the various shock-producing procedures with which we were familiar only two seemed likely to yield to such standardization, namely, partial temporary occlusion of the aorta or of the inferior vena cava. Exposure of the abdominal viscera has been so used by Mann (8), but we doubt if such damage can be uniformly applied. Other forms of mechanically produced tissue damage are very uncertain in their effects or cannot be inflicted aseptically (9); massive doses of adrenalin do not always cause death through shock; and it is still very questionable whether asphyxia (10) or fat embolism (11) bring on shock properly so-called, either clinically or experimentally.

#### THE STANDARD DAMAGE

After a few trials we decided to use as the damage partial occlusion of the vena cava (12). The exact procedure finally adopted as the result of a number of preliminary trials was as follows: Under morphine and ether anesthesia, and under strict asepsis, a clamp, adjustable by means of a finely threaded screw, is placed on the inferior vena cava of the dog between the diaphragm and the liver, and the vein is so compressed as to attempt to hold the arterial pressure at 40 mm. Hg. for a period of 2 hours 15 minutes. The ether is discontinued after applying the clamp, and owing to the apathetic condition of the animal, it need not be administered again.

As a rule the arterial pressure can thus be brought down to 40 mm. Hg. at once and held there. But sometimes the arterial pressure at

first does not fall to 40. In such instances, however, the pressure as a rule tends to fall, and usually reaches 40 mm. Hg. in the course of a few minutes, when the clamp can be opened and adjusted so as to hold the pressure at 40. Occasionally, however, the pressure falls quite slowly and in a few instances it has failed to reach the level of 40 mm. Hg., even by the end of the 2 hour and 15 minute clamping period. We have in several of the latter instances determined the position of the clamp by post-mortem examination and invariably have found that it was occluding the cava. Nevertheless, recognizing the danger of including in the series cases in which the position of the clamp may have been faulty, we have once and for all excluded all instances in which the pressure by the end of the period had failed to fall to within a millimeter or two of 40. It may be added that the pressure is just as apt to fail to fall when the initial arterial pressure is high as when it is low.

Experience has indicated that the cases in which difficulty is experienced in lowering the pressure are rather more apt to recover from the effects of the caval occlusion than cases in which the pressure can be brought down to 40 mm. Hg. at once. Indeed, in one or two instances the former, after removal of the clamp, have recovered without exhibiting any of the evidences of shock. The more favorable course of such cases seems capable of two explanations: either *a*, the high pressure protects the heart and medullary centers from the damage we have reason to believe (2) is done by the long-continued low pressure of the clamping period; or *b*, the high pressure of the clamping period is the result of an unusually good collateral circulation which protects the posterior parts of the body, as well as the anterior, from the effects of the occlusion. But whatever the explanation, the fact remains that failure of the pressure to fall seems to favor recovery. Some rule must, therefore, be made that will take this fact into account. We feel that recovery would be favored but little, if any, where the period during which the clamp is tight and the pressure above 40 mm. Hg. does not exceed 30 minutes. This arbitrary decision is applied in the statistical treatment of the experiments; it will be seen that the exclusion of such of these cases as recover does not materially alter the results.

When the clamp is removed the arterial pressure rises with a bound, sinks again almost at once, usually to somewhere between 50 to 75 mm. Hg., and then mounts slowly. Experience has shown that the animal will die if now the pressure begins to fall consistently, even

though slowly, before 2 hours have elapsed.<sup>2</sup> This outcome we have not succeeded in preventing by any of the forms of treatment we have employed. We therefore exclude from the series those cases in which the pressure begins to fall consistently before 2 hours have elapsed, and treatment is not begun until this two hour period of observation has passed.

One case (no. 215, table 7) has not been accepted although, strictly speaking, it is not excluded by this rule. The pressure in this case did not fall during, though it began to fall immediately after, the conclusion of the observation period and before treatment was begun. This has not occurred in any other case. Furthermore, the pressure at its highest did not get above 64 mm. Hg., a level which is 6 mm. below the lowest maximum observed in the whole series of 168 cases.

If the animal does not die within 48 hours, timed from the beginning of the clamping period, as a consequence of the damage done through clamping the cava, very marked improvement almost invariably is apparent particularly as regards the state of apathy.

In eight instances, however, animals that had shown this improvement were found dead unexpectedly, seven at the close, the eighth at the beginning, of the third day. Such animals, obviously, had recovered from the immediate effects of the injury, but died of other causes. They are, therefore, included in the category of those that recovered from shock. Our reasons for thus treating these cases will be discussed later.

A number of animals have become unavailable on account of certain accidents. Thus, animals that have died within 2 days and in which, at autopsy, extensive abdominal hemorrhage was found, must be discarded, for here the animal has had to contend not alone with the

<sup>2</sup> There has been but one exception to this rule, and that exception was not a clean-cut one. In the case of dog 208 (see table 3) after removing the clamp the pressure rose from the minimum of 55 mm. Hg. to a maximum of 97 mm. Hg. in the course of 27 minutes, but during the next 30 minutes it fell gradually to 80. Toward the close of the 2-hour observation period it began to rise, reaching 90 mm. Hg. 2 hours 10 minutes after removing the clamp. The animal's pressure, followed 2 hours longer, continued to rise, eventually reaching 95 mm. Hg. This animal recovered. It can scarcely be said that in this case the pressure began to fall *consistently* before the close of the 2-hour period; or as a matter of fact it eventually began to rise. It should be added that this was also a *cardiac* case (see below) and in the effort to carry the animal through the clamping period of 2 hours 17 minutes, it twice was necessary to partially open the clamp, once for a period of 8 minutes and once for a period of 2 minutes.

effects of the temporary anemia of the clamping period, but also with the effects of the hemorrhage. In most instances this hemorrhage was unavoidable and usually due to tearing of the liver through tension exerted by the clamp on an unusually broad suspensory ligament.

Cases exhibiting slight abdominal hemorrhage at autopsy have been included in the series. The decision as to whether a case that is on the border line is to be included in, or excluded from, the series must of necessity be arbitrary. We have therefore presented the results obtained after excluding every case showing abdominal hemorrhage of any degree whatever. It will be seen that this way of viewing the data affects them quite distinctly. Abdominal hemorrhage, it might be added, has been no more frequent in treated than in untreated animals. This fact is mentioned in order to allay any suspicion that the hemorrhage might be a consequence of treatment. We add, also, that we have observed no evidence of a tendency to bleed other than that attributable to the rise in pressure produced by the injection.

The control series, that is, the series of animals used for the purpose of determining the mortality rate from the standard damage, includes 23 "acceptable" cases. The acceptable group, to repeat, is made up by excluding cases in which the arterial pressure began to fall before the 2 hours had elapsed after removing the clamp, or in which at autopsy considerable abdominal hemorrhage was found. It is seen (table 1) that 52 per cent of the animals recovered. This method of doing damage, therefore, does not meet one of the conditions required of the ideal test; the damage inflicted does not just kill all the animals. We were unable, however, to come any closer to the ideal. When the clamping period was prolonged beyond 2 hours 15 minutes or when the arterial pressure was held below 40 mm. Hg., so as to get every untreated animal to die subsequently, treatment was absolutely without avail. The method adopted of preparing animals for treatment is not, therefore, as satisfactory as we had hoped to evolve, but though the method is not ideal, we felt convinced that by using a sufficiently large number of animals and by basing conclusions only on decided results, it would suffice our purposes. On the other hand the method has the advantage of indicating, through variations in the death rate, deleterious action as well as beneficial action.

Analysis of the control series, as well as of the pre-treatment stage of the several treated series, indicated the possibility of using in the interpretation of the results of treatment other data than those furnished by the number of deaths. Thus, it is seen that the span of



life in the fatal cases varies. It is obvious that if any particular form of treatment were injurious, its deleterious action might manifest itself not alone in an increase in the number of deaths but also in a shortening of the duration of the anti-mortal stage. As there were long periods during the night when the animals were not under observation, and as most of the animals, especially those dying within

TABLE 1

*Summary of mortality statistics*

| TREATMENT                            | TOTAL NUMBER OF ANIMALS |          |        | ACCEPTABLE CASES |        | DEATHS WITHIN 48 HOURS |          | AFTER EXCLUDING SLIGHT HEMORRHAGE |      |                        |        | DEATHS WITHIN 24 HOURS |                                   | EXCLUDING FATAL CARDIAC CASES |                        | EXCLUDING CASES 30' + ABOVE 40 MM. Hg. THAT LIVED |      |                        |
|--------------------------------------|-------------------------|----------|--------|------------------|--------|------------------------|----------|-----------------------------------|------|------------------------|--------|------------------------|-----------------------------------|-------------------------------|------------------------|---|------|------------------------|
|                                      |                         |          |        |                  |        | Number                 | Per cent | Number                            | Left | Deaths within 48 hours |        | Total                  | After excluding slight hemorrhage | Left                          | Deaths within 48 hours |   | Left | Deaths within 48 hours |
|                                      | Number                  | Per cent | Number | Per cent         | Number |                        |          |                                   |      | Per cent               | Number |                        |                                   |                               | Per cent               | Number  |      | Per cent               |
|                                      | 1                       | 2        | 3      | 4                | 5      | 6                      | 7        | 8                                 | 9    | 10                     | 11     | 12                     | 13                                | 14                            | 15                     | 16  | 17   |                        |
| Controls.....                        | 40                      | 23       | 11     | 48               | 4      | 19                     | 7        | 36.9                              | 4    | 1                      | 22     | 10                     | 45                                | 18                            | 11                     | 61.2  |      |                        |
| 6 in 2 per cent gum-bicarbonate..... | 27                      | 20       | 9      | 45               | 3      | 17                     | 6        | 35.3                              | 2    | 2                      | 19     | 8                      | 42                                | 16                            | 9                      | 56.3  |      |                        |
| 25 and 5 per cent gum-bicarbonate..  | 27                      | 16       | 9      | 56               | 2      | 14                     | 7        | 50.0                              | 8    | 6                      | 15     | 8                      | 53                                | 17                            | 9                      | 53.0  |      |                        |
| 25 and 18 per cent gum-glucose.....  | 43                      | 20       | 9      | 45               | 4      | 16                     | 5        | 31.3                              | 1    | 0?                     | 18     | 7                      | 39                                | 19                            | 9                      | 47.4  |      |                        |
| 25 in 18 per cent gum-glucose.....   | 33                      | 21       | 5      | 24               | 4*     | 18 or 17               | 2 or 1   | 11.0 or 5.9                       | 3*   | 0                      | 20     | 4                      | 20                                | 16                            | 5                      | 31.3  |      |                        |

\* One of these cases had no abdominal hemorrhage, but the pleura contained a large amount of bloody exudate and the liver was the seat of a chronic process.

24 hours, succumbed during the night, we find it impossible in most instances to give the duration of life in hours. It is, however, possible to accurately divide the fatal cases into those that die within 24 hours and those that die within 48 hours, and this we have done.

The arterial pressure also furnishes information of value in the analysis of the results obtained. Tables 2 and 3 show that in the control series the average initial arterial pressure is very much higher

in the case of the animals that died (109.8 mm. Hg.) than in the case of those that lived (89.4 mm. Hg.). Excepting one group, this is true also of the groups of animals that were subsequently treated. This observation can mean but one thing, namely, that a high initial pressure prejudices the animal's chances of recovery from the effects of holding its pressure down to 40 mm. Hg. for a fixed period. To what this may be due is a question we need not attempt to answer here.

TABLE 2  
*Summary of average pressures*

|                                       |                  |                        | Number of cases | DIED             |                        |                           |                      | RECOVERED OF SHOCK |                  |                        |                           |                      |
|---------------------------------------|------------------|------------------------|-----------------|------------------|------------------------|---------------------------|----------------------|--------------------|------------------|------------------------|---------------------------|----------------------|
|                                       | Initial pressure | Pressure after 2 hours |                 | Initial pressure | Pressure after 2 hours | Pressure before treatment | Pressure 1st reading | Number of cases    | Initial pressure | Pressure after 2 hours | Pressure before treatment | Pressure 1st reading |
|                                       | 1                | 2                      | 3               | 4                | 5                      | 6                         | 7                    | 8                  | 9                | 10                     | 11                        | 12                   |
| Controls.....                         | 99.6             | 102.9                  | 11              | 109.8            | 102.4                  | 105.0                     | 105.0                | 12                 | 89.4             | 103.4                  | 109.2                     | 109.2                |
| 6 in 2 per cent gum-bicarbonate.....  | 100.9            | 101.7                  | 9               | 107.9            | 101.9                  | 102.6                     | 111.1                | 11                 | 93.9             | 101.5                  | 103.7                     | 113.4                |
| 25 and 5 per cent gum-bicarbonate.... | 94.2             | 103.2                  | 9               | 98.8             | 98.3                   | 99.8                      | 113.0                | 7                  | 89.7             | 108.1                  | 109.3                     | 118.0                |
| 25 and 18 per cent gum-glucose.....   | 105.0            | 105.0                  | 9               | 106.6            | 97.5                   | 99.6                      | 115.1                | 11                 | 103.4            | 112.6                  | 115.1                     | 131.2                |
| 25 in 18 per cent gum-glucose.....    | 103.1            | 103.1                  | 5               | 102.0            | 100.2                  | 103.0                     | 118.0                | 16                 | 104.2            | 106.0                  | 106.8                     | 117.6                |
| Averages.....                         | 100.6            | 103.2                  |                 | 105.0            | 100.1                  | 101.2                     | 114.3*               |                    | 96.1             | 106.3                  | 108.7                     | 120.0*               |

\* Controls not included.

The important point is that on the average the animal with the high initial arterial pressure evidently is handicapped.

On the other hand it is seen, and for this purpose again all of the groups are available, that by the end of the 2-hour period of observation the arterial pressure of the cases that live, on the average, reaches a level considerably above the initial, whereas the average arterial pressure of the cases that die usually falls short of reaching the initial level. Presumably, therefore, speaking generally, a high post-decompression pressure favors recovery from the effects of clamping.

## Control series

## TREATMENT OF TRAUMATIC SHOCK

127

| RECOVERED OF SHOCK |                  |   |                                     |          |                                |   |               |                  |   |                                     |          |  |
|--------------------|------------------|---|-------------------------------------|----------|--------------------------------|---|---------------|------------------|---|-------------------------------------|----------|--|
| Serial number      | Initial pressure | Pressure end of ob-<br>servation period | Last reading                        |          | Slight abdominal<br>hemorrhage | Remarks   | Serial number | Initial pressure | Pressure end of ob-<br>servation period | Last reading                        |          | Remarks  |
|                    |                  |   | Time after<br>observation<br>period | Pressure |                                |   |               |                  |   | Time after<br>observation<br>period | Pressure |  |
| 101                | 125.0            | 120.0                                   | 21                                  | 125      | —                              | 8' above 40                                       | 93            | 105.0            | 104.0                                   | 20                                  | 108.0    | 86' above 40   |
| 96                 | 120.0            | 111.0                                   | 31                                  | 112      | —                              | 7' above 40                                       | 100           | 102.0            | 122.0                                   | 59                                  | 128.0    | Blood pressure at 29   |
| 98                 | 120.0            | 90.0                                    | 25                                  | 93       | —                              |   | 35            | 95.0             | 100.0                                   | 0                                   | 100.0    | hours; 105 mm. Hg;<br>animal seemed well;<br>killed              |
| 103                | 118.0            | 108.0                                   | 15                                  | 112      | +                              | Died within 24 hours                              | 120           | 95.0             | 100.0                                   | 0                                   | 100.0    | 13' above 40   |
| 92                 | 115.0            | 98.0                                    | 12                                  | 99       | —                              |   | 208           | 95.0             | 88.0                                    | 95                                  | 95.0     | 4' above 40. Cardiac;<br>clamped 2 hours 17',<br>partly open 10' |
| 111                | 110.0            | 100.0                                   | 12                                  | 103      | —                              | Cardiac; clamped 2<br>hours 16', partly<br>open ? | 102           | 90.0             | 100.0                                   | 23                                  | 110.0    |  |
| 62                 | 110.0            | 84.0                                    | 33                                  | 90       | —                              |   | 115           | 90.0             | 100.0                                   | 24                                  | 112.0    |  |
| 117                | 102.0            | 95.0                                    | 0                                   | 95       | +                              | Died within 24 hours.                             | 207           | 85.0             | 115.0                                   | 125                                 | 135.0    |  |
| 104                | 100.0            | 125.0                                   | 26                                  | 130      | +                              | 19' above 40                                      | 37            | 80.0             | 90.0                                    | 17                                  | 100.0    |  |
| 106                | 98.0             | 110.0                                   | 0                                   | 110      | +                              | Died within 24 hours                              | 119           | 80.0             | 98.0                                    | 18                                  | 102.0    | 47' above 40   |
| 116                | 90.0             | 85.0                                    | 34                                  | 86       | —                              | Died within 24 hours.<br>15' above 40             | 107           | 78.0             | 110.0                                   | 0                                   | 110.0    | 44' above 40   |
|                    |                  |   |                                     |          |                                |   | 118           | 78.0             | 108.0                                   | 7                                   | 110.0    | 75' above 40   |
| Ave.               | 109.8            | 102.4                                   |                                     | 105      |                                |   | Ave.          | 89.4             | 103.4                                   |                                     | 109.2    |  |

TABLE 4  
Six per cent gum acacia in 2 per cent  $\text{NaHCO}_3$ , 12 cc. per kilo

| DIED          |                  |                                    |   |                       |                 |   |                             |               |                  | RECOVERED OF SHOCK                 |   |                       |                 |   |  |
|---------------|------------------|------------------------------------|---|-----------------------|-----------------|---|-----------------------------|---------------|------------------|------------------------------------|---|-----------------------|-----------------|---|--|
| Serial number | Initial pressure | Pressure end of observation period | Time between observation period and treatment | Treatment             |                 | Remarks   | Slight abdominal hemorrhage | Serial number | Initial pressure | Pressure end of observation period | Time between observation period and treatment | Treatment             |                 | Remarks                                       |  |
|               |                  |                                    |   | Pressure at beginning | Pressure at end |   |                             |               |                  |                                    |   | Pressure at beginning | Pressure at end |   |  |
|               | mm.              | mm.                                | min.  | mm.                   | mm.             |   |                             |               | mm.              | mm.                                | min.  | mm.                   | mm.             |   |  |
| 134           | 135.0            | 117.0                              | 13  | 120.0                 | 135.0           | Died within 24 hours, 8' above 40                               | —                           | 130           | 130.0            | 120.0                              | 0   | 120.0                 | 130.0           | 134' above 40                                 |  |
| 126           | 130.0            | 93.0                               | 1   | 93.0                  | 96.0            | 6' above 40   | +                           | 52            | 110.0            | 108.0                              | 0   | 108.0                 | 120.0           | 11' above 40                                  |  |
| 139           | 120.0            | 100.0                              | 0   | 100.0                 | 110.0           | 77' above 40  | —                           | 60            | 105.0            | 75.0                               | 46  | 85.0                  | 100.0           |   |  |
| 127           | 120.0            | 95.0                               | 17  | 95.0                  | 100.0           | 12' above 40. Cardiac; clamped 2 hours 15'; clamp partly open ? | —                           | 129           | 100.0            | 116.0                              | 7   | 116.0                 | 120.0           | 134' above 40                                 |  |
| 123           | 110.0            | 115.0                              | 3   | 115.0                 | 120.0           |   | +                           | 61            | 100.0            | 84.0                               |   | 85.0                  | 100.0           |   |  |
| 58            | 101.0            | 100.0                              |   | 100.0                 | 110.0           |   | —                           | 142           | 100.0            | 130.0                              | 41  | 130.0                 | 140.0           | Died end of 3rd day. No autopsy               |  |
| 63            | 90.0             | 94.0                               | 43  | 95.0                  | 103.0           | Died within 24 hours  | —                           | 141           | 83.0             | 87.0                               | 38  | 90.0                  | 100.0           |   |  |
| 143           | 85.0             | 108.0                              | 16  | 110.0                 | 120.0           |   | —                           | 137           | 80.0             | 120.0                              | 24  | 120.0                 | 120.0           | 56' above 40. Died end of 3rd day. No autopsy |  |
| 125           | 80.0             | 95.0                               | 2   | 95.0                  | 106.0           |   | +                           | 50            | 80.0             | 85.0                               | 0   | 85.0                  | 110.0           | 30' above 40                                  |  |
|               |                  |                                    |   |                       |                 |   |                             | 133           | 75.0             | 97.0                               | 21  | 100.0                 | 110.0           |   |  |
|               |                  |                                    |   |                       |                 |   |                             | 57            | 70.0             | 94.0                               | 21  | 98.0                  | 98.0            | 16' above 40                                  |  |
| Ave.          | 107.9            | 101.9                              |   | 102.6                 | 111.1           |   |                             | Ave.          | 93.9             | 101.5                              |   | 103.7                 | 113.4           |   |  |



TABLE 5  
*Twenty-five per cent gum acacia (Na), 5 cc. per kilo and 5 per cent NaHCO<sub>3</sub>, 5 cc. per kilo*

| RECOVERED OF SHOCK |                  |                                    |  |                       |                 |  |               |                  |                                    |
|--------------------|------------------|------------------------------------|--|-----------------------|-----------------|--|---------------|------------------|------------------------------------|
| Serial number      | Initial pressure | Pressure end of observation period | Time between observation and treatment | Treatment             |                 | Remarks  | Serial number | Initial pressure | Pressure end of observation period |
|                    |                  |                                    |  | Pressure at beginning | Pressure at end |  |               |                  |                                    |
| mm.                | mm.              | min.                               | mm.                                    | mm.                   | mm.             |  |               | mm.              | mm.                                |
| 175                | 125.0            | 11.0                               | 14                                     | 115.0                 | 127             | 13' above 40. Died within 24 hours                                 | 166           | 100.0            | 102.0                              |
| 176                | 114.0            | 129.0                              | 4                                      | 130.0                 | 150             |  | 182           | 98.0             | 117.0                              |
| 170                | 110.0            | 95.0                               | 9                                      | 95.0                  | 120             | Died within 24 hours   | 165           | 95.0             | 112.0                              |
| 41*                | 105.0            | 91.0                               | 14                                     | 92.0                  | 100             | Died within 24 hours   | 172           | 95.0             | 110.0                              |
| 159                | 100.0            | 112.0                              | 51                                     | 112.0                 | 120             | Died within 24 hours   | 171           | 90.0             | 109.0                              |
| 169                | 95.0             | 112.0                              | 26                                     | 117.0                 | 124             | Died within 24 hours   | 163           | 60.0             | 107.0                              |
| 49†                | 90.0             | 83.0                               | 3                                      | 85.0                  | 100             | Died within 24 hours   | 43            | 100.0            | 100.0                              |
| 164                | 75.0             | 76.0                               | 22                                     | 76.0                  | 82              | Died within 24 hours   |               |                  |                                    |
| 174                | 75.0             | 75.0                               | 8                                      | 76.0                  | 94              | Died within 24 hours. Cardiac; clamped 2 hours 15', partly open 5' |               |                  |                                    |
| Ave.               | 98.8             | 98.3                               |  | 99.8                  | 113             |  | Ave.          | 89.7             | 108.1                              |
|                    |                  |                                    |  |                       |                 |  |               |                  | 109.3                              |
|                    |                  |                                    |  |                       |                 |  |               |                  | 118                                |

\* 4 per cent NaHCO<sub>3</sub> combined with acacia. Some blood removed for Hb. determination.

† 5 per cent NaHCO<sub>3</sub> combined with acacia.

*Cardiac cases.* A certain number of animals die of, or are threatened by, cardiac failure during the period of occlusion. We have not had an opportunity to study this condition carefully. It comes on as a rule during the first hour of the clamping period. Its imminence is indicated by an irregularity in the force and rhythm of the pulse as registered by the arterial manometer. If, when this is noted, the clamp on the cava is cautiously opened and the heart massaged through the thorax, the pulse in the course of a few minutes may become perfectly regular again. Once the heart has thus recovered, the arterial pressure often can again be held down by the clamp for the rest of the clamping period and the heart, as a rule, will not again become irregular. But quite as often opening the clamp and allowing the pressure to rise at the time cardiac failure becomes imminent does not help matters. Sometimes, indeed, it seems to do harm: the irregularity increases, the pressure falls and the heart stops.

The further course of the cases showing these cardiac disturbances, but which, through temporary opening of the clamp, can be carried to the period of observation, seems to indicate, however, either that the damage that led to the cardiac disturbance was not altogether transient, or that the effect upon the heart is a measure of the damage the occlusion of the cava has inflicted on the body generally. If we include in this analysis only those cases in which it was possible to carry out the clamping stage as usual, with the exception of the few minutes during which it was necessary to open the clamp in order to save the heart, and exclude the cases of marked abdominal hemorrhage, the whole series contains 22 cardiac cases. In 10 or 11 of these cases the arterial pressure after removing the clamp began to fall before the 2-hour period of observation had elapsed. In accordance with the rule, all of these died excepting the one of this character previously referred to. Twelve of the cases belong, therefore, to the series from which we deduce the results of applying measured damage. Six of these died within 48 hours despite the fact that their clamping period is usually somewhat curtailed by the period during which the heart is being given a chance to recover.

That the cardiac cases are somewhat handicapped is indicated also by the behavior of the arterial pressure during the observation period. While the average arterial pressure of all of the cases that died of clamping the cava was 100.1 mm. Hg. at the close of the 2-hour period, of all that recovered, 106.3 mm. Hg., the average of the 6 cardiac cases that died was 85.2, of the 6 that recovered, 100.7. This last group

includes one case (see table 6) whose arterial pressure rose to 135 mm. Hg., the highest pressure reached after 2 hours in the whole series of 219 animals. If we exclude this exceptional case, the average pressure of the cardiac cases that recovered becomes 93.8 mm. Hg. These cases, therefore, have to contend with a circulation that is inferior to that of the general run to a degree that is indicated by a lower arterial pressure amounting to from 12 to 15 mm. Hg.

It seems only fair that these facts should be taken into account in considering the effects of treatment. With this end in view, we have included in the statistics the figures obtained after excluding the cardiac cases that died. It will be seen, however, that the general relation of the several groups to each other is not materially altered by this method of presenting the results.

#### THEORY OF THE TREATMENTS

The object we had in mind when this investigation was begun was to determine the relative efficacy of solutions containing gum acacia and a crystalloid in the prevention of death due to trauma. The number of solutions that might have been studied in such an investigation was almost limitless. Inasmuch as there were reasons for reaching an early decision, and inasmuch as it was realized that large numbers of animals would be required to satisfactorily test each solution, it was necessary to reduce the number tested to the smallest that might suffice to indicate the advantages and disadvantages of the different types of combinations.

At the time, a solution containing bicarbonate as the crystalloid had been recommended for use at the front by the English Committee on Shock and Allied States (13). By starting the tests with this first Bayliss solution, therefore, we felt it would be possible at one and the same time to test the claims made for this solution and to determine the efficacy not alone of a solution practically isotonic as regards both colloid and crystalloid but also one containing an alkali which also was being recommended for use in the treatment of shock (14). The results obtained with such a solution could then be compared with the results obtained with a hypertonic solution of the same ingredients. For reasons that will become evident, solutions containing glucose instead of bicarbonate were also tested.

1. *Gum acacia, 6 per cent, in  $\text{NaHCO}_3$ , 2 per cent.* This solution was made up about as described in the private report of the Special

TABLE 6  
*Twenty-five per cent gum acacia (Ca) 5 cc. per kilo and 18 per cent glucose, 5cc. per kilo*

| RECOVERED OF SHOCK |                  |   |   |                          |                    |  |               |                  |   |   |                          |                    |   |
|--------------------|------------------|---|---|--------------------------|--------------------|--|---------------|------------------|---|---|--------------------------|--------------------|---|
| Serial number      | Initial pressure | Pressure end of ob-<br>servation period | Time between ob-<br>servation period<br>and treatment | Treatment                |                    | Remarks  | Serial number | Initial pressure | Pressure end of ob-<br>servation period | Time between ob-<br>servation period<br>and treatment | Treatment                |                    | Remarks   |
|                    |                  |   |   | Pressure at<br>beginning | Pressure at<br>end |  |               |                  |   |   | Pressure at<br>beginning | Pressure at<br>end |   |
| 150                | 130.0            | 90.0                                    | 19  | 94.0                     | 122.0              | 5' above 40. Cardiac;<br>clamped 2 hours 16',<br>clamp partly open 19' | 153           | 125.0            | 127.0                                   | 30  | 132.0                    | 150.0              |   |
| 90                 | 125.0            | 107.0                                   | 6   | 107.0                    | 130.0              | 10' above 40   | 72            | 120.0            | 118.0                                   | 2   | 118.0                    | 135.0              | 38' above 40  |
| 79                 | 116.0            | 99.0                                    | 10  | 100.0                    | 118.0              |  | 77            | 120.0            | 93.0                                    | 5   | 96.0                     | 118.0              | Died end of 3rd day.<br>Peritonitis; cardiac;<br>clamped 2 hours 15';<br>clamp partly open 7' |
| 61                 | 115.0            | 84.0                                    | 25  | 87.0                     | 90.0               | 25' above 40   | 54            | 105.0            | 90.0                                    | 28  | 97.0                     | 105.0              | Died end of 3rd day.<br>No autopsy  |
| 89                 | 110.0            | 105.0                                   | 10  | 105.0                    | 118.0              | Died within 24 hours   | 69            | 105.0            | 118.0                                   | 13  | 120.0                    | 137.0              |   |
| 76                 | 100.0            | 108.0                                   | 22  | 110.0                    | 120.0              |  | 75            | 104.0            | 130.0                                   | 12  | 130.0                    | 130.0              | 10' above 40  |
| 156                | 100.0            | 114.0                                   | 14  | 115.0                    | 128.0              | 134' above 40  | 136           | 100.0            | 130.0                                   | 28  | 135.0                    | 165.0              |   |
| 73                 | 85.0             | 95.0                                    | 27  | 100.0                    | 118.0              |  | 147           | 100.0            | 108.0                                   | 4   | 108.0                    | 125.0              |   |
| 81                 | 78.0             | 75.0                                    | 24  | 78.0                     | 92.0               | Cardiac; clamped 2 hours<br>20', clamp partly open<br>5'               | 80            | 98.0             | 98.0                                    | 6   | 98.0                     | 120.0              | 11' above 40  |
|                    |                  |   |   |                          |                    |  | 155           | 85.0             | 124.0                                   | 22  | 124.0                    | 128.0              | Died in 51½ hours.  |
|                    |                  |   |   |                          |                    |  | 88            | 75.0             | 103.0                                   | 9   | 108.0                    | 130.0              | Peritonitis; cardiac;<br>clamped 2 hours 20';<br>clamp partly open 5'                         |
| Ave.               | 106.6            | 97.5                                    |   | 99.6                     | 115.1              |  | Ave.          | 103.4            | 112.6                                   |   | 115.1                    | 131.2              |   |



Committee on Shock and Allied Conditions of November 9, 1917. Enough sodium bicarbonate to make a 2 per cent solution was added to a 6 per cent solution of gum acacia in water. The solution was heated and the precipitated  $\text{CaCO}_3$  centrifuged off. The solution was then heated again, and if another precipitate formed, this again was removed, this process continuing until heating the mixture sufficiently long to sterilize it no longer gave rise to a precipitate.

The only essential divergence from the method of preparation recommended by the English Committee consisted in not conducting the heating of the solution in a sealed vessel. Our method of preparation probably converted most of the bicarbonate into carbonate. In this respect, however, the difference between the two methods is only relative. For even admitting that in the process of preparation as recommended by the English Committee the bicarbonate is dissociated only to the extent that occurs ordinarily at room temperature and in atmospheric air, only a certain proportion of it would be present as such (15), and the dissociation would tend to go further at the time the solution, heated for injection, is exposed to atmospheric air. This change in the bicarbonate can not be avoided by sterilizing the dry substance by dry heat, for unless this is done in the presence of  $\text{CO}_2$  under tension, the bicarbonate at the sterilizing temperature also would be converted into carbonate practically completely (15). As a matter of fact, unless solutions containing bicarbonate are kept exposed to  $\text{CO}_2$  at a relatively high tension at the time they are being injected more or less carbonate will always be present.

In order to understand the action of this solution on the blood volume and on the alkali reserve, it is necessary to know approximately the salt concentration remaining in solution after precipitating the lime and after boiling. The amount of calcium contained in gum acacia seems to vary within very wide limits. If we take the figure given by Bayliss, namely, 2.23 per cent (16), the minimum amount of  $\text{NaHCO}_3$  left in the 2 per cent solution after adding it to the 6 per cent solution of gum acacia would make a 1.9 per cent solution. If boiling converts all of the bicarbonate into carbonate, the minimum salt concentration would be 1.5 per cent  $\text{Na}_2\text{CO}_3$ . The freezing point of the solution, if the conversion into carbonate were complete, would be about  $-0.6^\circ\text{C}$ . or  $-0.77^\circ\text{C}$ . for the unconverted bicarbonate; in other words the freezing point of the solution as injected would probably be somewhat lower than that of the blood plasma ( $-0.56^\circ\text{C}$ .). When these experiments were done, we were of the opinion that this first

Bayliss solution was isotonic with respect to the blood colloids. We now know that it is slightly hypertonic in this respect also. But the differences from isotonicity are probably not so large as to be of any particular significance (7).

The dose of the solution used in these experiments, 12 cc. per kilo of body weight, is somewhat larger than that (500 cc.) recommended by the English Committee for use in man, which for a 60 kilo man is 8.3 cc. per kilo. The report does not specify the rate at which this dose is to be administered. We used the larger dose because it was about the amount needed to restore the blood of a shocked animal to its normal concentration (7), to say nothing of the amount needed to make good the stagnated blood. The larger dose was used also in order that the alkali would come closer to the amount needed to combat the average acidosis that develops in experimental shock. But even in the larger dosage the alkali, presumably, was not nearly sufficient for this purpose (7). The time taken to administer our dose averaged 29 minutes.

2. *Gum acacia, 25 per cent, and sodium bicarbonate, 5 per cent.* The solutions for this treatment were made up and administered in several ways. At first enough  $\text{NaHCO}_3$  to make a 5 per cent solution was dissolved in the 25 per cent gum, the solution heated and the precipitate centrifuged off. The gum, however, turned deep brown in color, especially during sterilization, and while we had no evidence that the colored gum was harmful, it was deemed best not to use it. The next method consisted in adding a very slight excess of the bicarbonate to the gum solution, applying heat and then centrifuging off the precipitated  $\text{CaCO}_3$ . The solution was then neutralized carefully with  $\text{HCl}$  and sterilized in sealed centrifuge tubes. If, during sterilization, a new precipitate formed this was thrown down in the centrifuge. This solution of sodium acacia was decanted into a sterile burette as needed and the bicarbonate, dry sterilized, added in sufficient amount to make a 5 per cent solution of bicarbonate. In by far the largest number of experiments, 5 cc. per kilo of the sterile, neutralized sodium acacia, made as described above, were first given and immediately followed by a solution made by adding dry-sterilized bicarbonate to sterile water in sufficient amount to make a 5 per cent solution of  $\text{NaHCO}_3$ . From time to time the proportions and the dosage were varied (see table 5) but never by enough to materially alter the conditions as here described. The administration of the acacia solution

occupied about a half-hour, of the carbonate solution about 23 minutes. The rate of injection of the solution, calculated as bicarbonate, was therefore 0.65 gram per kilo and hour. The rate recommended by the English Committee (17) was about 0.95 gram per kilo and hour in the form of a 4 per cent solution of bicarbonate made up, presumably as we have made our solution.

The dosage we employed was based upon the view (16) that 7 per cent gum acacia was isotonic with respect to the colloids of the blood and that, therefore, the water the colloid theoretically could hold would be about the amount needed to restore the blood volume of shocked animals to normal. Newer estimations of the osmotic pressure of gum acacia (4), (7) indicate, however, that the figure used by us was somewhat too low. The bicarbonate was added in sufficient amount to draw into the circulation approximately the amount of water the gum on the basis of the available osmotic data was capable of holding. The amount of alkali administered was greater in the proportion of 25 to 21 than in the dose of the solution consisting of 6 per cent gum and 2 per cent bicarbonate. In neither case, though, was it present in amounts large enough to balance, by chemical means, at least, the acidosis usually present at the time the injection is begun (7).

3. *Gum acacia, 25 per cent, and glucose, 18 per cent*, were given in two ways. In one series the 25 per cent gum acacia was given first and was followed immediately by the 18 per cent glucose. The carefully filtered gum solution was sterilized under pressure in stoppered centrifuge tubes and in case a turbidity developed the solution was cleared in the centrifuge. In order to avoid the change in the glucose solution that is produced by sterilization at high temperatures, as indicated by the yellow color that develops, the glucose solutions at first were sterilized by pasteurization. Subsequent experience showed, however, that the autoclaved glucose is not injurious; therefore, the latter method of sterilization was finally adopted. The dose of each of the solutions was 5 cc. per kilo of body weight. The injection of the gum again occupied about 30 minutes. The glucose injection averaged about 19 minutes. The latter, therefore, was given faster than the tolerant rate of 5 cc. per kilo and hour. The rationale of this dosage was the same as that underlying the acacia-carbonate dosage. The acacia was in such a concentration that through its osmotic tension it could easily hold the water which the 18 per cent solution of glucose would draw into the circulation. The effect of





[illegible]

this combination upon the blood volume of shocked animals has not been determined. It has, however, been shown in normal animals that the results obtained with it are in general accord with theory (7).

4. *Gum acacia, 25 per cent, and glucose, 18 per cent, in combination.* In the foregoing series the acacia and glucose were given in the succession, in the dosage and at the rates described in order to make the conditions comparable with those obtaining in the series that received 25 per cent gum and 5 per cent bicarbonate solutions. The two latter substances could not conveniently be given in one solution because, as has been said, the gum acacia in strongly alkaline solution seemed to be considerably changed during sterilization. And it seemed advisable to give the aftercoming crystalloid as rapidly as was safe in order to bring rapidly into the circulation the water needed to dilute the gum acacia. There were reasons for suspecting, however, that the combined administration of acacia and crystalloid might have some advantages over their successive administration. For until this water was added, the circulation of blood would be slowed by the increase in its viscosity produced by the strong gum solution. By combining the glucose with the gum this necessity no longer existed and it then was possible to inject the glucose at a slower rate, one which in normal animals does not lead to glycosuria (18). In this series of experiments, therefore, the gum and the glucose were combined in one and the same solution. The method of making up this solution has been described in another place (19). It was injected at the rate of 5 cc. per kilo and hour; the glucose, therefore, entered the circulation at the subtolerant rate of 0.9 gram per kilo and hour.

The effect of the injection of solutions 3 and 4 upon the alkali reserve has not been determined. According to Macleod and Hoover (20), alkaline glucose injections increase the amount of lactic acid in the blood. Acid glucose solutions, they showed, do not have this effect. The present solution, through the reaction of the acacia, is faintly acid: presumably, therefore, there would be no acid formed through the process described by Macleod and Hoover.

#### STATISTICS

Excluding, as has been explained, the cases in which the arterial pressure began to fall consistently before the close of the 2-hour period of observation, it is seen (tables 1, 2, 3) that of the 23 acceptable cases composing the control series, 11, or 48 per cent, died within 48 hours,

4 of these, or 17.4 per cent of the group, within 24 hours. In the series treated with the combination of 6 per cent gum acacia and 2 per cent sodium bicarbonate (tables 1, 4) there are 20 acceptable cases of which 9, or 45 per cent, died within 48 hours, and 1 of these, or 5.0 per cent of all the acceptable cases, within 24 hours. In the series receiving in succession 25 per cent gum acacia and 5 per cent sodium bicarbonate (tables 1, 5) there are 16 acceptable cases; 9, or 56 per cent, died within 48 hours; and 8 of these, or 50 per cent of all in the group, died within 24 hours. It was so obvious that this treatment was doing harm that it was not regarded as necessary to accumulate as many cases as we have in the other groups. The group receiving 25 per cent gum acacia and 18 per cent glucose in succession (tables 1 and 6) comprises 20 acceptable cases, 9 of which, or 45 per cent, died within 48 hours, and of these 1, or 5.0 per cent of all the cases, within 24 hours. And, finally, the series receiving the gum and glucose in combination (tables 1 and 7) contains 21 acceptable cases: 5 of these, or 24 per cent, died within 48 hours, and 3 of these, or 14.3 per cent of the whole number, within 24 hours.

The two most obvious results coming of this comparison of the several groups are *a*, the unfavorable showing of the series receiving the 25 per cent gum acacia and 5 per cent sodium bicarbonate in succession, as regards both total mortality and duration of life of the fatal cases; and *b*, the favorable showing of the series receiving 25 per cent gum in combination with 18 per cent glucose as regards total deaths. In the matter of one day deaths, the latter series is only slightly better than the control series and is not so favorable as the series that received the 6 per cent gum in 2 per cent bicarbonate or 25 per cent gum and 18 per cent glucose. The number of one day deaths in these three series is so small, however, that a difference of one case in either direction would practically have eliminated all differences. The high 24 hour mortality of the series receiving 25 per cent gum and 5 per cent bicarbonate cannot, however, be accounted for in this way. This treatment unquestionably does harm.

As has been said, a high arterial pressure at the end of the observation period seems to favor recovery. The average pressures of all of the groups at this time (table 2) are, however, so nearly alike that it is not necessary to make any allowance for such effect as this factor may have.

From the cases that died and are otherwise acceptable have been excluded, as has been explained above, the cases in which considerable

abdominal hemorrhage is found at autopsy. The necessity for excluding such cases is obvious. On the other hand, as has been said, the decision as to whether the hemorrhage is marked or slight rests upon a judgment which often it is difficult to make. In order to take this into account we give in columns 6, 7, 8 and 9 of table 1 the statistics as obtained by excluding all of the fatal cases in which at autopsy even a small amount of bloody fluid was found in the abdomen. It is seen that the relative number of hemorrhage cases in each group is practically the same, demonstrating again its accidental origin. It follows that when they are excluded the relative mortality rate is not markedly affected (column 9); the differences already presented (column 5) are merely somewhat emphasized.

In columns 10 and 11 (table 1) it is seen that with the exception of two groups, practically all of the deaths occurring within 24 hours were complicated by slight hemorrhage. The exceptions are in the case of the groups receiving bicarbonate and seem to emphasize from still another aspect the deleterious effects of alkali. Set over against this is the fact that all of the one day deaths occurring in the series receiving 25 per cent gum acacia in 18 per cent glucose were complicated by the slight hemorrhage; there are left in this group altogether only 1 or 2 deaths if those with slight hemorrhage are excluded.

It has been pointed out that, in general, cases with high initial arterial pressures are more apt to die than cases with low initial pressures. On this basis it is seen (table 2, columns 1 and 9) that the group with the best initial chance, namely, those treated with 25 per cent gum acacia and 5 per cent sodium bicarbonate, actually is the one that did the worst; while the group with the worst chances in this respect, namely, the one receiving 25 per cent gum acacia followed by 18 per cent glucose, did quite as well as any; and that the treatment that gave the best results, namely, the 25 per cent gum acacia in 18 per cent glucose, was tried out on a group whose initial chances were not particularly favorable.

The results obtained by excluding, for reasons already given, the cases in which the pressure failed to fall to 40 mm. Hg. within 30 minutes after applying the clamp to the cava and lived are seen in columns 15, 16, 17 of table 1. These exclusions cause no important changes in the relative positions of the several groups. The treatment consisting of 25 per cent gum acacia and 5 per cent sodium bicarbonate still gives the worst result; that consisting of 25 per cent gum acacia in 18 per cent glucose the best. The group receiving 6 per cent gum



acacia in 2 per cent sodium bicarbonate gains a little, that receiving 25 per cent gum acacia followed by 18 per cent glucose, somewhat more, on the control group.

Finally, we take into consideration the handicap of the cases in which the heart during the clamping period threatened to fail, by calculating the results after excluding those of these cases that die. Columns 12, 13, 14 (table 1) show that this precaution also changes the results but little. Its main effect is to improve slightly the results of the administration of the solutions containing glucose.

It has already been stated that the series of acceptable cases includes some 12 so-called heart cases, and that of these 6 recovered of shock. It is interesting to add here that of the 6 that recovered, 5 were in the series that were treated with glucose, namely, 2 in the group receiving acacia and glucose in succession, and 3 in the group receiving the solutions simultaneously. One was in the control group. While the number of cardiac cases is not sufficiently large to permit of any definite conclusion, it certainly is suggestive that with but one exception, all of the cardiac cases that recovered of shock had received intravenously a substance, glucose, which is known to affect favorably the contraction of heart muscle.

Thus far we have confined ourselves to the action of the solutions on the state that develops, to all intents and purposes, immediately after unclamping the vena cava, that is, on shock. It has already been stated that 8 of the animals that had shown marked improvement from this first state died rather unexpectedly, for the most part, toward the end of the third day. The possible significance of such deaths did not at first occur to us and as a consequence we have autopsy notes on only four of the cases. These four presented signs of peritonitis ranging from a fibrinous deposit on the peritoneum to a sanguino-purulent exudate. The fatal issue in these instances, therefore, can be attributed neither to shock nor to a late deleterious action of the injected solutions. The other four cases not autopsied were scattered among three of the groups. In view of the relatively small number of these cases—4 out of the 100 that were acceptable—and in view of the regular presence of peritonitis in the four cases that came to autopsy, and in view, furthermore, of the many other possible causes of death, even if peritonitis were excluded, it certainly seems unnecessary to attribute these deaths to a delayed action of the injected solutions. It might be added that the incidence of peritonitis was not high when it is taken into account that the peritoneum was open for

$2\frac{1}{4}$  hours at a time when its resistance, owing to the slowing of the circulation, must have been quite low, and that there was the possibility of infection through the walls of the gastro-intestinal tract damaged by the long-lasting ischemia.

#### DISCUSSION

The foregoing analysis shows that no matter how the statistics are viewed, the relative efficacy of the solutions tried is — 25 per cent calcium acacia in 18 per cent glucose > 25 per cent gum acacia followed by 18 per cent glucose = or > 6 per cent sodium acacia in 2 per cent sodium bicarbonate = or > untreated controls > 25 per cent sodium acacia followed by 5 per cent sodium bicarbonate.

In attempting to account for these results it should first be recalled that in shocked animals all of the solutions that have been tested are capable of restoring the blood volume and the blood pressure and of maintaining these to practically the same degree under similar circumstances. Considering the end in view it certainly seems justifiable to assume that these responses are desirable. The fact, therefore, that some of the solutions, 6 per cent gum acacia in 2 per cent sodium bicarbonate and 25 per cent gum acacia followed by 18 per cent glucose, for example, effect but little, if any, reduction in the death rate of traumatized animals, may mean that these solutions are not entirely innocuous and that the harm they do just balances the good that results from the improvement they effect in the circulation. There can, of course, be no doubt but that the treatment in which the 25 per cent sodium acacia is followed by 5 per cent sodium bicarbonate is harmful. Is it possible to ascertain from the limited data available wherein this harmfulness consists?

Gum acacia in 6 per cent solution we believe is wholly innocuous. This is proved by our own experience. Even a 25 per cent solution of gum acacia in the dosage which we have used is quite innocuous to normal animals. When such a solution is given rapidly to dogs until the blood plasma becomes a 10 per cent solution of gum acacia no obvious symptoms develop (22). Czerny (23) found the maximal non-lethal dose for cats and rabbits to be 4.66 grams per kilo. This is almost four times the amount contained in the dose we give in shock and in the concentration (24 per cent) used by Czerny, would convert the plasma of these animals into an 8 per cent solution of gum. And Czerny did not even use aseptic precautions. The innocuousness of the hypertonic

solution is illustrated also by the biological tests we have been making of the solution consisting of 25 per cent gum in 18 per cent glucose made up for the treatment of shock in man (19). Each batch of this solution is tested by injecting in the course of a half-hour 10.0 cc. per kilo into an animal previously bled to the extent of more than 3 per cent of its body weight. In none of the animals so treated has there been the slightest evidence of harm.

There is no question, though, but that concentrated gum in the dosages we have employed may do harm if it is injected *rapidly* into an animal about to die of shock. Under these circumstances, in our experience, the heart occasionally has stopped as though it had fibrillated. Presumably, this is the result of the slowing of the circulation that comes of the high viscosity of the injected gum solution. At any rate this harmful action cannot be attributed to the osmotic properties of the gum, for nothing of the kind is seen when the gum in equally hypertonic solution is injected more slowly, not even when it is in combination with hypertonic glucose. Nor can it be due to any chemical action of the gum, for inasmuch as the injected gum remains for some time in circulation, there is finally just as much of it in circulation when it is injected slowly as when it is injected rapidly; yet, under the former circumstances stoppage of the heart does not occur. To be sure, calcium acacia has been used almost exclusively in these tests; but as regards osmotic (4) and viscosity (16) factors, as these affect the blood, the sodium and calcium acacias are presumably practically alike. In the course of our experiments we have given large doses of both of these acacias and have not been able to satisfy ourselves that there is any difference in their action. Furthermore, if sodium acacia acts deleteriously through chemical means, the results obtained from the injection of the sodium acacia in combination with isotonic bicarbonate should have been almost as bad as the results of the injection of the 25 per cent solution followed by the hypertonic carbonate, for the amount of gum given with the former (0.72 gm. per kilo) is not very much smaller than the amount given with the latter (1.25 gm. per kilo). The difference in the results following the injection of these two solutions, though, is quite striking.

The result of this analysis, as far as it has gone, is to indicate, therefore, that it is neither the sodium acacia *per se* that does the harm nor its hypertonicity, but rather the alkali that is injected with or after it, to a certain extent also the high viscosity of the more concentrated gum solution that lasts until it is diluted by its own osmotic action

and by that of the crystalloid subsequently injected. It is not necessary to conclude at this juncture, however, that the bicarbonate is injurious by virtue of its alkalinity. It may be that saline crystalloids are undesirable in the treatment of shock, possibly because they accumulate and disturb the salt balance of the body.

It was considerations of this character that led us, when it was found that the gum in combination with bicarbonate is without value in the treatment of shock, to try out glucose as the crystalloid, especially in view of the indications obtained by one of us in collaboration with Woodyatt (24) that it ameliorates the symptoms of experimental shock better than do salines.

The series of experiments in which the glucose replaced the bicarbonate but comparable in every other respect save the nature of the gum, clearly demonstrated the superiority of the glucose as a means of drawing the water to the gum. Yet this succession of gum and glucose was without any marked effect upon the death rate due to the measured trauma. The next step was to eliminate the period of increased viscosity of the blood by injecting the gum and the crystalloid simultaneously. It was by this method that the maximum saving of life was effected.

It is conceivable that this beneficial action is due not alone to the osmotic action of the glucose but also to some specific action it may exert. With regard to the relative merits of salt and glucose as a means of bringing water into the circulation, it may first be pointed out that there is relatively little reason, other than osmotic, for administering salts in shock, for there is, so far as is known, no salt deficit in that state. Neither is there any lack of glucose (14). But glucose has the advantage over most other crystalloids that might be used for this purpose, that through oxidation and polymerization, the organism can quickly put it out of the way after it has accomplished the purpose of bringing water to the gum acacia. However this may be, there is abundant evidence in the literature indicating that glucose of itself acts beneficially in clinical shock and allied conditions. Woodyatt, with Sansum and Wilder, obtained favorable results from sustained injection of glucose in hypertonic solution in two cases having features of shock (24). Hypertonic glucose has been found to be of benefit in pneumonia (25) and in a number of other conditions.

There are a number of ways in which glucose might prove of functional value to the traumatized organism. The fact that in shock there is no deficiency of blood sugar does not necessarily mean that



in this respect the administration of glucose would prove futile. The beneficial results said to come of feeding sugar to soldiers on the march (26), for instance, can not be attributed to any deficiency of glucose in the blood (27). Whatever the mechanism of the relief from fatigue may be, it seems likely that the same or a similar mechanism might work to relieve shock when glucose is administered. It is well known that glucose in excess of the normal blood content is oxidized in the tissues (28), and that it is used as a food when it is introduced into the system parenterally (29). Glucose also improves and sustains the beat of the perfused heart (30), (31), as well as the contractions of other types of muscle (32), (33). In this connection it is of interest to recall that of the six cardiac cases that recovered of shock, five were in the groups that received glucose.

Over and above all of the considerations that have thus far been discussed, there was another which held out hopes that hypertonic solutions might prove of value in the treatment of shock. Eyster and Wilde (34) have found that certain crystalloids in hypertonic solution, glucose among others, cause an immediate increase in cardiac output and a vasodilatation. These actions apparently are independent of the resultant hydremia; indeed, they seem to be specific. But whatever may be the cause, it is obvious that these are exactly the responses best calculated to improve the circulation in shock.

It will be noted that we have not tested the efficacy of blood in the treatment of shock. One reason for not doing so was the number of animals that would have been required, while another was the fact that our animals were not suffering from any loss of blood. When, as in pure shock, there is no actual loss of blood, but rather a concentration of the blood and a crowding of the small veins and capillaries with corpuscles, the introduction of more blood, unless it promptly betters the condition, would seem to be contra-indicated. At least this is the inference one is led to draw from the effects of the injection of blood into normal animals. Starling points out (35) that under these circumstances the blood fluids do not remain long in the vessels but pass into the lymphatics, leaving behind the corpuscles and a certain proportion of the proteins of the plasma. This concentration of the blood raises its viscosity and tends to embarrass the circulation; there is produced a state of affairs similar to the one we are trying to combat in shock. For this reason it is conceivable that blood plasma may have some advantages over whole blood in the treatment of non-hemorrhagic shock. Furthermore, the injection of blood even in suit-

able cases is by no means entirely free of danger (36). It is, therefore, of interest to recall in this connection that blood transfusion is not essential to recovery even from the severest acute hemorrhage, if only the blood bulk can be restored in other ways (37). The present investigation possibly indicates that a restoration of blood volume in which the tissues are made to participate through osmotic action is more efficacious than one effected through the injection of the full amount of fluid needed to make good the reduction.

*Results in man.* After we had convinced ourselves through the animal experiments described in this paper that hypertonic gum and glucose are not alone innocuous if given slowly, but actually save a certain number of animals from death by trauma, we began the use of this solution in the treatment of shock in man. The results obtained in eleven cases have been recently described in another place (19). It need only be stated here that they fully confirm the conclusions reached in this paper.

#### SUMMARY

Of animals traumatized by holding the arterial pressure down to 40 mm. Hg. for 2 hours and 15 minutes by partially occluding the inferior vena cava—

48 per cent die within 48 hours.

When treated with:

- |   |                                  |
|---|----------------------------------|
| a. 6 per cent gum in 2 per cent sodium bicarbonate 12 cc. per kilo of body weight                   | 45 per cent die within 48 hours. |
| b. 25 per cent gum followed by 5 per cent sodium bicarbonate, of each 5 cc. per kilo of body weight | 56 per cent die within 48 hours. |
| c. 25 per cent gum followed by 18 per cent glucose, of each 5 cc. per kilo of body weight           | 45 per cent die within 48 hours. |
| d. 25 per cent gum in 18 per cent glucose, 5 cc. per kilo of body weight and hour                   | 24 per cent die within 48 hours. |

Not only is the death rate increased by treatment *b*, but death occurs earlier.

These results are taken to indicate that bicarbonate and the high viscosity of a strong gum solution, are somewhat harmful, at least, in

traumatized animals; that the harmfulness of the strong, viscid gum can be avoided in part through the osmotic action of hypertonic glucose subsequently injected, but not by bicarbonate; and that when the hypertonic gum and the hypertonic glucose are given simultaneously and slowly so as to avoid altogether the period during which the high viscosity of the gum is hampering the circulation, a maximum saving of life can be effected. The beneficial results presumably are due to the internal transfusion (38) effected by the hypertonic solutions, to the maintenance of the increased blood volume through the colloidal and possibly other properties of the gum acacia, to the action of the hypertonic solution on the heart and blood vessels, and to the specific action of glucose on nutrition in general and on that of the heart muscle in particular.

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## STUDIES IN SECONDARY TRAUMATIC SHOCK

### VII. NOTE ON THE ACTION OF HYPERTONIC GUM ACACIA AND GLUCOSE AFTER HEMORRHAGE<sup>1</sup>

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The injuries that lead to shock in man almost always are accompanied by more or less hemorrhage. When the hemorrhage is so large as to be dangerous of itself the patient suffers not alone from the immediate and direct consequences of the blood loss but according to prevailing opinion also runs the risk of passing in time into shock properly so-called, possibly, as a consequence of the action of the slowed blood stream upon the peripheral vessels (1).

However this may be, it soon became obvious to us after we had acquired some experience with clinical shock that the surgeon not infrequently is called upon to treat cases with the symptoms of shock in which the extent of hemorrhage is unknown, or in which it is known to be dangerous but for which blood for transfusion is not immediately available. And the question arose in this connection, is it justifiable to employ the hypertonic gum-glucose solution in such instances? Experience gained through the use of the isotonic gum-saline solution in the British Army has led Bayliss to conclude (2) that this substitute for blood is especially useful in those cases in which shock is complicated with a certain amount of hemorrhage. We have not the slightest doubt but that this is true also of the hypertonic gum-glucose solution (3). But the question that concerns us here is not this, but rather, is a hemorrhage that is so extreme as to be apt to prove rapidly fatal as the result of the blood loss itself, a contraindication to the use of the hypertonic gum-glucose solution?

<sup>1</sup> Reported to the Committee on Shock of the National Research Council, July, 1918.

## EXPERIMENTAL

The method employed in seeking the answer to this question has been to select two animals as nearly alike as possible as regards weight, vigor and breed, to bleed them both, under ether, to about the same extent in proportion to body weight, and then to give to one of each of the couples 5 cc. of the 25 per cent gum acacia, 18 per cent glucose solution per kilo of body weight in the course of an hour. The usual aseptic precautions were observed. It will be noted in table 1 that the extent of the hemorrhage was not always exactly the same in the two animals of a couple. This came about because of differences in the reaction of the animals to the hemorrhage; the development of threatening symptoms, such as an exceedingly low arterial pressure or marked slowing of the heart rate, indicating that further hemorrhage would probably prove immediately fatal, sometimes forced us to desist in the withdrawal of blood in one or other of the two animals before the intended amount had been drawn.

*First hemorrhage.* In the dog, according to Fredericq (4), a loss of blood amounting to 2.3 to 4.5 per cent of the body weight is dangerous, the loss of over 4.5 to 5.0 per cent, fatal. Our aim was to remove from the animal by a rapid hemorrhage an amount of blood that would just about prove fatal. As will be seen in table 1, the amount at first

TABLE 1  
*Showing the amount of blood drawn in per cent of body weight*

|                        | GROUP 1 |       | GROUP 2 |       | GROUP 3 |       |
|------------------------|---------|-------|---------|-------|---------|-------|
|                        | Dog 1   | Dog 2 | Dog 3   | Dog 4 | Dog 5   | Dog 6 |
| First hemorrhage.....  | 4.41    | 3.96  | 4.45    | 4.64  | 4.80    | 4.80  |
| Second hemorrhage..... | 3.16    | 3.19  | 3.14    | 3.13  | 2.67    | 2.60  |
| Total.....             | 7.57    | 7.15  | 7.59    | 7.77  | 7.47    | 7.40  |

removed ranged between 3.96 and 4.8 per cent of the body weight, yet not one of the animals succumbed, treated or untreated.

In the case of group 1 (dogs 1 and 2, fig. 1, dots) more blood was removed from the test animal (dog 1, large dots) than from its control (dog 2, small dots); the fall in pressure in the case of the control was so profound that the hemorrhage had to be discontinued for fear of immediately killing the animal. This animal had the lower initial pressure. The pressure of the test animal in the course of 1 hour 30 minutes came back to within 10 mm. of its initial value; whereas the pressure of the untreated animal remained 37 mm. Hg. below its normal.

The initial conditions were reversed in the case of the second group (dogs 3 and 4, crosses). For the reasons mentioned above, it was not possible in this case to remove from the test animal (dog 3, large crosses) quite as much blood as from its control (dog 4, small crosses). The difference, though, was inconsiderable. The arterial pressure of the test animal was affected by the hemorrhage much more profoundly than that of the control. The arterial pressure of the treated animal eventually rose to 88 mm. Hg. (the initial was 87); of the untreated animal to 100 mm. Hg. (the initial was 95).

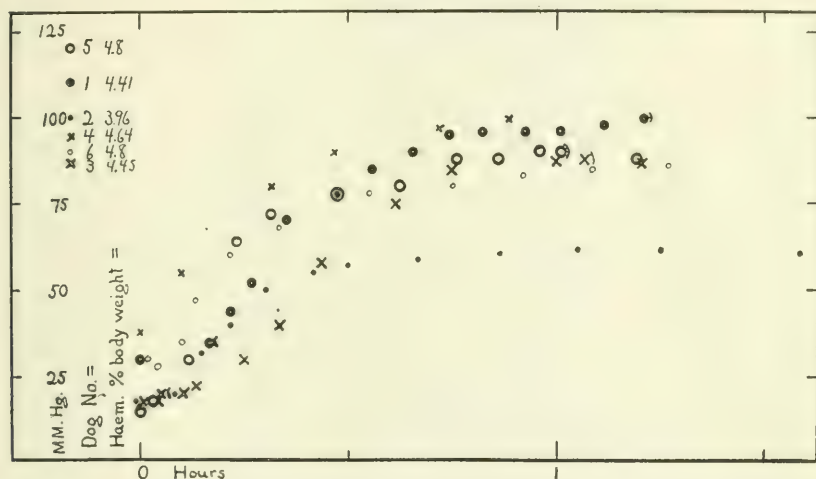


Fig. 1. Chart showing the effects of the first hemorrhage. Dog 1 (treated) ●; dog 2 (control) •; dog 3 (treated) X; dog 4 (control) x; dog 5 (treated) ○; dog 6 (control) o. The injection period is the time included in each case between the parentheses.

In the case of the third couple (dogs 5 and 6, circles) the hemorrhages were exactly equal in amount; 4.8 per cent of the body weight was removed. The arterial pressure of the test animal fell lower as the result of the hemorrhage than that of the control. The arterial pressure of the control animal rose to within 4 mm. of the initial level, that of the treated animal to within 30 mm., but the pressure of the treated animal at the end was a bit above that of the untreated animal.

The only conclusion these observations justify is that the injection of the gum-glucose solution did not prejudice the chances of recovery from the effects of a severe hemorrhage. Whether any of the treated

TABLE 2

|  | GROUP 1          |                |                   |                | GROUP 2          |                |                   |                | GROUP 3          |                |                   |                |
|--|------------------|----------------|-------------------|----------------|------------------|----------------|-------------------|----------------|------------------|----------------|-------------------|----------------|
|  | First hemorrhage |                | Second hemorrhage |                | First hemorrhage |                | Second hemorrhage |                | First hemorrhage |                | Second hemorrhage |                |
|  | Dog 1, test      | Dog 2, control | Dog 1, test       | Dog 2, control | Dog 3, test      | Dog 4, control | Dog 3, test       | Dog 4, control | Dog 5, test      | Dog 6, control | Dog 5, test       | Dog 6, control |
| Initial pressure.....                                      | (1)              | (2)            | (3)               | (4)            | (5)              | (6)            | (7)               | (8)            | (9)              | (10)           | (11)              | (12)           |
| Hemorrhage per cent body weight.....                       | 110              | 100            | 105               | 85             | 87               | 95             | 90                | 105            | 120              | 90             | 88                | 96             |
| Pressure at end of hemorrhage.....                         | 4.41             | 3.96           | 3.16              | 3.19           | 4.45             | 4.61           | 3.14              | 3.13           | 4.8              | 4.8            | 2.6               | 2.6            |
| Pressure at or corresponding with beginning of injury..... | 30               | 18             | 28                | 30             | 18               | 38             | 78                | 74             | 15               | 30             | 76                | 80             |
| Present at or corresponding with end of injury.....        | 35               | 34             | 41                | 38?            | 20               | 45             | 70                | 79             | 18               | 28             | 65                | 77?            |
| Highest pressure.....                                      | 100              | 62             | 95                | Dead           | 88               | 100?           | 95                | Dead           | 90               | 84             | 78                | 85             |
| Pressure last reading.....                                 | [100]*           | [62]           | 96                | 45             | [88]             | [100]?         | 95                | 79             | [90]             | 86             | 79                | 88             |
|  | [100]            | 60             | [96]              | 0              | 87               | [100]?         | 0                 | 0              | 88               | [86]           | 73                | 77             |

\*Bracketed pressure readings are the same as the unbracketed ones next above.



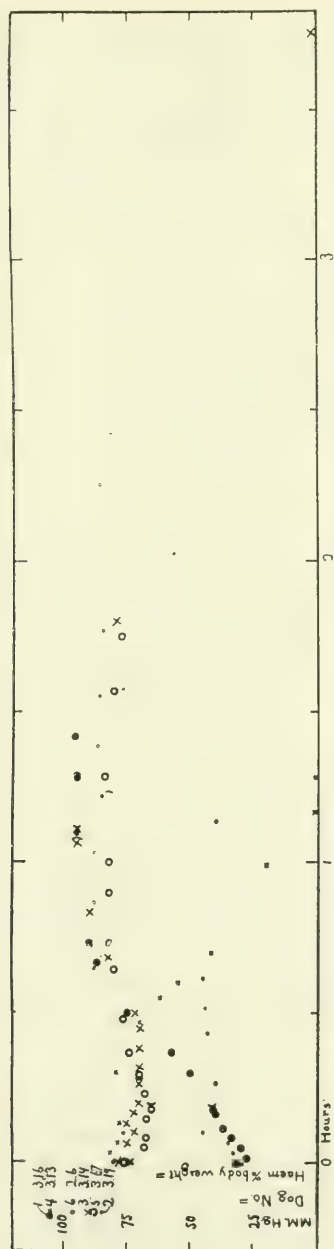


Fig. 2. Chart showing the effects of the second hemorrhage. Designations as in figure 1.

animals would have died had the solution not been administered cannot be determined.

*Second hemorrhage.* Some two or three days subsequent to the experiments above described each couple was again bled, the amounts taken this time varying between 2.6 per cent and 3.2 per cent of the body weight (see table 1). The amount of blood removed was almost exactly alike in the two animals of each of the couples. The total of the two hemorrhages ranged between 7.15 and 7.77 per cent of the body weight.

In the case of group 1 (dogs 1 and 2, fig. 2, dots) the immediate effect of the hemorrhage was to carry the arterial pressure down to the same level but the absolute drop was greater in the case of test animal. After treatment (dog 1, large dots) the pressure rose to 96 mm. Hg.; this animal recovered. The pressure of the untreated animal rose only to 45 mm. Hg., then fell and the animal died 1 hour 33 minutes after the hemorrhage.

The animals of group 2 (dogs 3 and 4, crosses) were bled to the same extent (3.14 to 3.15 per cent of the body weight). Their pressures as a consequence did not fall very low, but they fell to about the same level. After the hemorrhage for about 36 minutes the pressure of the control animal practically maintained its level while that of the test animal slowly fell. Then the pressure of the control animal began to fall and this animal died 2 hours 20 minutes after the hemorrhage. At the same time under the influence of the injection the decline of the arterial pressure in the treated animal was converted into a rise; it mounted until, by the end of the injection period, it was 5 mm. Hg. above the initial level, but then it began to fall, though slowly, and the animal died 4 hours 30 minutes after the hemorrhage.

Group 3 was bled to the extent of 2.67 per cent (treated) and 2.6 per cent (untreated) of the body weight. The initial pressure of the former was somewhat lower than that of the latter and during and subsequent to the hemorrhage it fell the lower. Both of these animals survived.

#### DISCUSSION

This analysis of the experimental data shows that the animal of each couple that received the solution did quite as well as, or better than, its control. In thus stating our conclusion we do not desire to give the impression that we are advocating the use of hypertonic gum-glucose solution in the treatment of pure and immediately dangerous hemor-

rhage, though we do believe that when blood is not instantly available it is safe and perhaps advisable to give the gum-glucose solution pending the obtaining of blood. This opinion is based not alone on the results of the present experiments, but also upon our clinical experience (see cases IV, IX and X in our paper on the treatment of shock in man (3) ).

Penfield (5) has compared the effect of the following solutions, *a*, 0.9 per cent sodium chloride; *b*, 6.0 per cent gum acacia and 2.0 per cent sodium bicarbonate; *c*, 6.0 per cent gum acacia and 6.0 per cent glucose,—injected into animals after so bleeding them as to hold the arterial pressure down to 40 mm. Hg. for periods ranging between 60 and 89 minutes. The volume of solution injected equalled the amount of blood drawn and varied (as we calculate it) between 4.15 and 5.05 per cent of the body weight. If we except three of his eleven cases, the amount removed and injected averaged 4.44 per cent and did not exceed 4.65 per cent of the body weight. So far as can be determined there were no untreated controls in his series. One of the three of his animals that received the sodium chloride solution died; the average hemorrhage amounted to 4.61 per cent of the body weight. The amount of blood withdrawn at the time of the first hemorrhage in our experiments averaged 4.51 per cent of the body weight and therefore was practically as large as in Penfield's and not a single animal died whether treated or not. There is, therefore, no necessity for concluding that the isotonic solution of sodium chloride in Penfield's hands accomplished any good.

Three of the four animals into which Penfield injected his gum-glucose solution died; the average hemorrhage was 4.29 per cent of the body weight. At this place we desire to correct the statement made by Penfield that he used "gum-glucose solution as recommended by Erlanger." Ours is hypertonic gum-glucose, not isotonic. Having removed this possibility of a misunderstanding, we may say that we are surprised not that three of the four animals treated with the weak gum-glucose solution died, but rather that one of them lived. For Penfield replaced approximately half of the blood in the body with the same amount of a solution which within a very few minutes, through oxidation and polymerization of the glucose, came to consist practically of a solution of 6.0 per cent gum in pure water. The effect that this must have had upon the tissues and the salt balance of the organism, unquestionably accounts for the results Penfield obtained.

The hypertonic gum-glucose solution, of course, is changed in the same way in the organism, but the dose we give, namely, 5 cc. per kilo an hour, is so small, and it is given so slowly, that such disturbance in salt balance as it may cause is negligible in comparison with that caused by the dose of 40 to 50 cc. per kilo employed by Penfield.

#### CONCLUSIONS

1. The use of hypertonic gum-glucose solution is not contra-indicated in the treatment of shock even when it is complicated by dangerous hemorrhage.

2. The fact that the hypertonic gum-glucose solution does not prejudice the recovery of animals from the effects of a hemorrhage that is apt to result fatally furnishes another proof of the innocuousness of this solution.

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## THE INFLUENCE OF OXYGEN ADMINISTRATION ON THE CONCENTRATION OF THE BLOOD WHICH ACCOMPANIES THE DEVELOPMENT OF LUNG EDEMA

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The enormous and rapid development of edema of the lungs which results from severe gassing of animals with the lung irritants used in warfare offers an unusual opportunity for studying the physiological effects accompanying this pathological condition. The rapidity with which the edema develops precludes the possibility of infection complicating the symptoms observed, and the condition which may develop after exposure to high concentrations of poisonous gas is so severe that the correlated symptoms can hardly be overlooked.

Loss of water from the blood is one of the most characteristic phenomena accompanying the development of edema of the lungs in animals gassed with lung irritants. A concentration of the blood becomes evident at about the time when the edema of the lungs can be first demonstrated. Thereafter the loss of water from the blood and the increase in severity of the edema run roughly parallel. The conclusion was therefore made that the two are interrelated and that the pouring of water into the lungs is the cause of the concentration of the blood.

Other considerations however make it necessary to proceed with caution before accepting this hypothesis. During the acute period after gassing there develops a deficiency of oxygen carried by the blood. Probably due to the poor aeration of the blood in the damaged lung the oxygen content of arterial and venous blood may drop to levels much below normal. The transport of oxygen to the tissues may be still further reduced by the decreased rate of blood flow with the probable result that the oxygenation of the tissues is seriously interfered with.

<sup>1</sup>Published with the permission of the American and British military authorities.

Physiologists have shown that muscle tissue imbibes water when supplied with insufficient oxygen. Based on this observation the hypothesis may be presented that the concentration of the blood is due, not primarily to the development of lung edema, but to the imbibition of water from the blood by the tissues which are not sufficiently oxygenated. To throw some light on the validity of this hypothesis the experiments reported below were carried out.

Goats were gassed with lethal concentrations of chlorpicrin. As soon as possible after gassing, half of the animals were fitted with masks and given oxygen continuously in known quantities by means of a Haldane oxygen apparatus.<sup>2</sup> The other animals were used as controls.

The hemoglobin content was used as an index of the concentration of the blood. Hemoglobin determinations were made frequently using blood obtained by pricking an ear vein. Blood from the heart punctures was also used. The Haldane method was used for the hemoglobin determinations.

When the concentration of the blood was sufficiently marked in the animals to which oxygen was being administered, heart punctures were made and the percentage saturation of the hemoglobin of the bloods from the right and left hearts was determined by means of Barcroft's differential blood gas apparatus.<sup>3</sup> Some difficulty was experienced in obtaining blood from the hearts of animals in which the lungs were large and edematous but the sample was considered satisfactory when it was obtained quickly and with little struggling on the part of the animal.

The protocols are given below as well as curves showing the concentration of the blood. In the curves, the percentage variations from the normal are plotted to make all of the curves directly comparable.

The maximum concentrations observed in the control animals varied from 30 per cent to 60 per cent above normal (average 43 per cent) while in the animals receiving oxygen the variation was from 28 per cent to 75 per cent above normal (average 48 per cent). It is apparent that, on the whole, the blood of animals which received oxygen con-

<sup>2</sup> The Haldane oxygen apparatus furnishes oxygen to a mask fitted with valves for incoming and outgoing air. When the mask is worn by the animal, breathing is easy and the air in the mask may be enriched by varying amounts of oxygen.

<sup>3</sup> We are indebted to Mr. Barcroft, Captain Dunn and Captain Peters for these data.

TABLE 1

*Chlorpicrin 1/8500 for 25 minutes (10.10 to 10.35 a.m.), August 19, 1918*

| GOAT 4537  | GOAT 4567  |
|--|--|
| 9.35 a.m. Hb. 80   | 10.00 a.m. Hb. 60  |
| 10.35 a.m. Gassed  | 10.35 a.m. Gassed  |
| 11.00 a.m. Continuous oxygen by mask,<br>1 liter per minute  | 11.00 a.m. Continuous oxygen by<br>mask, 1 liter per minute                              |
| 11.40 a.m. Hb. 79 (100 per cent)   | 11.50 a.m. Hb. 70 (117 per cent)   |
| 2.20 p.m. Hb. 85 (106 per cent)  | 2.40 p.m. Hb. 84 (140 per cent)  |
| 2.45 p.m. Continuous oxygen by<br>mask, 3 liters per minute  | 2.45 p.m. Continuous oxygen by<br>mask, 3 liters per minute                              |
| 4.25 p.m. Hb. 105 (131 per cent)   | 3.45 p.m. Hb. 105 (175 per cent).  |
| 4.45 p.m. Heart puncture. Arterial<br>blood 93 per cent saturated. Venous<br>blood 45 per cent saturated | Heart puncture. Venous blood 55<br>per cent saturated. Animal died<br>on table. L: H 8.0 |
| 5.45 p.m. Hb. 110 (137 per cent)   |  |
| 5.55 p.m. Died. L: H 8.2   |  |
| GOAT 4406 (CONTROL)  | GOAT 4542 (CONTROL)  |
| 9.45 a.m. Hb. 42   | 9.50 a.m. Hb. 74   |
| 10.35 a.m. Gassed  | 10.35 a.m. Gassed  |
| 12.10 p.m. Hb. 48 (114 per cent)   | 12.20 p.m. Hb. 76 (103 per cent)   |
| 2.30 p.m. Hb. 60 (143 per cent)  | 2.50 p.m. Hb. 88 (119 per cent)  |
| 3.00 p.m. Died. L: H 6.0   | 5.30 p.m. Hb. 96 (130 per cent).   |
|  | Found dead next morning. L: H 8.3  |

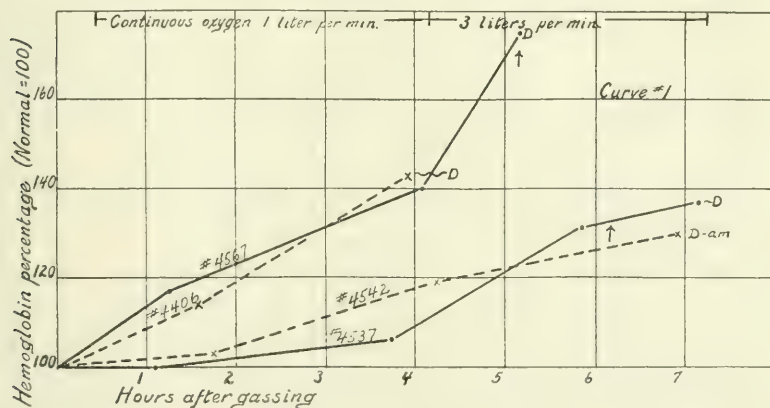


Fig. 1. Showing the concentration of the blood after gassing. Solid line: animals receiving extra oxygen. Arrows indicate time of heart puncture. No. 4567, venous blood 55 per cent saturated. No. 4537, arterial blood 93 per cent saturated; venous blood 45 per cent saturated. Dotted line: control animals.

TABLE 2

*Chlorpicrin 1/8500 for 30 minutes (9.30 to 10.00 a.m.), August 21, 1918*

| GOAT 4526  | GOAT 4446 (CONTROL)              |
|--|----------------------------------|
| 5.35 p.m. (8/19). Hb. 53   | 5.30 p.m. (8/19). Hb. 72         |
| 10.00 a.m. Gassed  | 10.00 a.m. Gassed                |
| 10.15 a.m. Continuous oxygen by mask, $1\frac{1}{2}$ liters per minute   | 12.15 p.m. Hb. 96 (133 per cent) |
| 12.00 m. Hb. 67 (126 per cent)   | 1.45 p.m. Hb. 118 (164 per cent) |
| 12.15 p.m. Continuous oxygen by mask, 4 liters per minute  | 2.00 p.m. Died.                  |
| 2.10 p.m. Hb. 70 (132 per cent)  |                                  |
| 3.20 p.m. Hb. 73 (138 per cent)  |                                  |
| 3.50 p.m. Hb. 76 (143 per cent). Heart puncture. Arterial blood 90 per cent saturated. Venous blood (obtained only after struggling) 20 per cent saturated |                                  |
| 4.15 p.m. Oxygen stopped   |                                  |
| 5.30 p.m. Hb. 76 (143 per cent)  |                                  |
| 6.05 p.m. Died. L: H 7.7   |                                  |

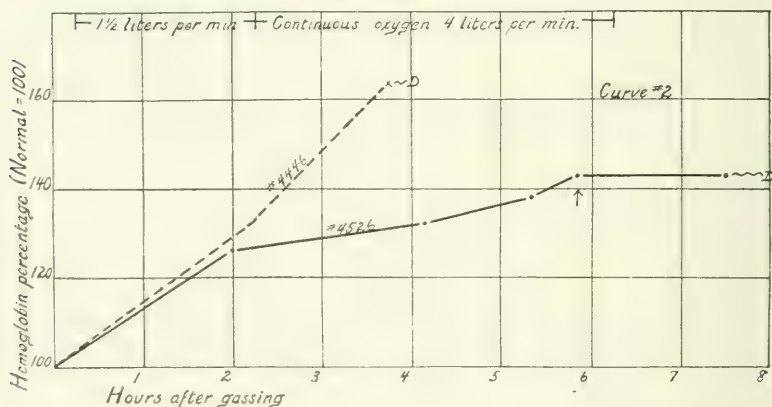


Fig. 2. Showing the concentration of the blood after gassing. Solid line: animals receiving extra oxygen. Arrow indicates time of heart puncture. No. 4526, arterial blood 90 per cent saturated. Dotted line: control animals.



TABLE 3

*Chlorpicrin 1/8500 for 25 minutes (9.16 to 9.41 a.m.), August 23, 1918*

| GOAT 4577   | GOAT 4631  |
|---|--|
| 5.50 p.m. (8/22). Hb. 60  | 5.45 p.m. (8/22). Hb. 58   |
| 9.41 a.m. Gassed  | 9.41 a.m. Gassed   |
| 9.55 a.m. Continuous oxygen by mask, 3 liters per minute  | 9.55 a.m. Continuous oxygen by mask, 3 liters per minute   |
| 10.23 a.m. Hb. 70 (117 per cent)  | 10.10 a.m. Hb. 54 (93 per cent)  |
| 12.12 p.m. Hb. 81 (135 per cent)  | 12.00 m. Hb. 58 (100 per cent)   |
| 2.35 p.m. Hb. 95 (158 per cent). Heart puncture. Both samples obtained only after struggling. Arterial blood 58 per cent saturated. Venous blood 4 per cent saturated | 2.50 p.m. Hb. 61 (105 per cent)  |
| 2.40 p.m. Died on table. L: H 7.4   | 5.00 p.m. Hb. 67 (116 per cent)  |
|   | 5.55 p.m. Hb. 69 (119 per cent)  |
|   | 6.20 p.m. Heart puncture. Arterial blood 95 per cent saturated. Venous blood about 50 to 60 per cent |
|   | 6.20 p.m. Hb. 74 (128 per cent). Died on table. L: H 5.7   |
| GOAT 4490 (CONTROL)   | GOAT 4457 (CONTROL)  |
| 9.41 a.m. Gassed  | 9.41 a.m. Gassed   |
| 10.43 a.m. Hb. 85   | 10.54 a.m. Hb. 94  |
| 12.30 p.m. Hb. 100 (118 per cent)   | 12.20 p.m. Hb. 96 (102 per cent)   |
| 2.15 p.m. Hb. 124 (146 per cent)  | 3.15 p.m. Hb. 106 (113 per cent)   |
| 2.20 p.m. Died. L: H 6.7  | 6.30 p.m. Hb. 124 (132 per cent). Found dead next morning. L: H 7.1                                  |

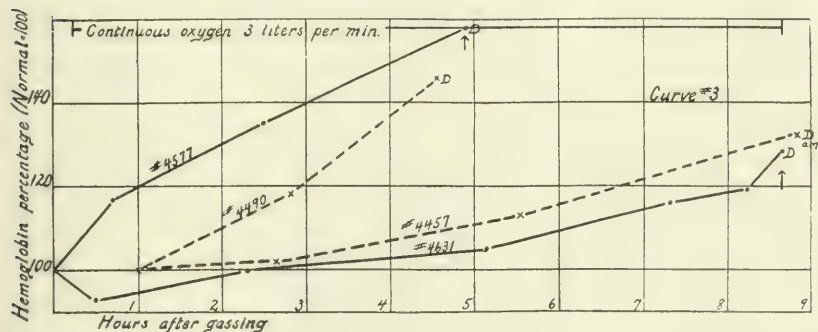


Fig. 3. Showing the concentration of the blood after gassing. Solid line: animals receiving extra oxygen. Arrows indicate time of heart puncture. No. 4577, both blood samples obtained only after struggling. No. 4631, arterial blood 95 per cent saturated; venous blood 50 per cent saturated.

centrated as rapidly and to as great an extent as that of the control animals.

In order to demonstrate the efficiency of the oxygen administration, samples of blood were taken from both sides of the heart at suitable intervals and analyzed for oxygen. In most of the experiments the venous and arterial blood samples were obtained from the heart without difficulty and contained hemoglobin which was normally saturated with oxygen. With the increased concentration of the hemoglobin the oxygen content of the blood was even above normal.

Occasionally the blood was obtained only after considerable struggling on the part of the animal so that the reduced oxygen content of such bloods was to be expected. These observations are reported here merely to make the experimental record complete, as obviously the low oxygen content of such bloods is without bearing on the present problem.

These experiments demonstrate that, by breathing oxygen-rich atmospheres, oxygen may be absorbed through the damaged and edematous lungs in quantities sufficient to maintain a practically normal level of oxygen in the arterial blood. The high saturation of the hemoglobin of venous blood with oxygen would seem to prove that the blood flow is sufficiently rapid to normally oxygenate the tissues. Nevertheless, in spite of the normal oxygenation of the tissues in the animals receiving oxygen, the blood concentrated as rapidly and to as great an extent as in the control animals. The conclusion therefore seems justifiable that the lack of oxygen in the tissues and consequent imbibition of water is not an important factor in causing the concentration of the blood in animals developing edema after being gassed with lung irritants.

An indication of the severity of the lung edema was obtained by comparing the weight of the lung to the weight of the heart at autopsy. The high lung to heart ratios obtained in practically all of the animals studied show that a severe grade of edema had already developed. The extent of the edema as indicated by this method was as great in the animals receiving oxygen as in the controls. Although the data are necessarily few, it is apparent that the efficient oxygenation of the lung tissue in the animals receiving oxygen failed to diminish the tendency for the development of the edema of the lungs.

With the enormous accumulation of fluid in the edematous lungs and the loss of water from the blood running roughly parallel, it is a tempting study to estimate even in a rough way the possible water

interchange. An attempt has been made with data which are more or less incomplete and with calculations involving gross errors but the relations are so striking that they are presented here (table 4). In the table are recorded data and calculations from animals in which the hemoglobin was not determined immediately before death but is estimated from the curve obtained from the various determinations. These estimated values are quite similar to average values obtained at death on other animals.

TABLE 4

*Comparison of calculated amounts of fluid lost from blood and extra fluid in the lungs in gassed animals*

| (1)<br>GOAT NUMBER | (2)<br>DATE | (3)<br>WEIGHT ANI-<br>MAL | (4)<br>WEIGHT HEART<br>AT DEATH | (5)<br>WEIGHT LUNG<br>AT DEATH | (6)<br>L:H | (7)<br>WEIGHT NOR-<br>MAL LUNG | (8)<br>LAST HEMO-<br>GLOBIN DE-<br>TERMINA-<br>TION | (9)<br>HEMOGLOBIN<br>AT DEATH | (10)<br>NORMAL BLOOD<br>VOLUME | (11)<br>BLOOD VOL-<br>UME AT<br>DEATH | (12)<br>FLUID LOST<br>FROM BLOOD<br>AT DEATH | (13)<br>EXTRA FLUID<br>IN LUNG |
|--------------------|-------------|---------------------------|---------------------------------|--------------------------------|------------|--------------------------------|---|-------------------------------|--------------------------------|---------------------------------------|--|--------------------------------|
|                    |             | kgm.                      | gram                            | gram                           |            | gram                           | per cent  | per cent                      | cc.                            | cc.                                   | cc.  | cc.                            |
| 3602               | 6/13        | 31.8                      | 157                             | 1472                           | 9.4        | 377                            | 163   | 200                           | 1750                           | 875                                   | 875  | 1095                           |
| 3638               | 6/12        | 29.5                      | 126                             | 623                            | 4.9        | 302                            | 125   | 140                           | 1625                           | 1160                                  | 465  | 321                            |
| 3787               | 6/20        | 16.8                      | 74                              | 566                            | 7.6        | 178                            | 128   | 145                           | 925                            | 638                                   | 287  | 388                            |
| 4098               | 7/ 4        | 35.0                      | 195                             | 1103                           | 5.7        | 468                            | 114   | 140                           | 1925                           | 1375                                  | 550  | 635                            |
| 4406               | 8/19        | 15.5                      | 85                              | 510                            | 6.0        | 204                            | 143   | 150                           | 850                            | 567                                   | 283  | 306                            |
| 3920               | 7/ 4        | 37.8                      | 200                             | 1700                           | 8.5        | 480                            | 180   | *                             | 2080                           | 1155                                  | 925  | 1220                           |
| 3600               | 6/12        | 25.5                      | 130                             | 849                            | 6.5        | 312                            | 154   | *                             | 1400                           | 910                                   | 490  | 537                            |
| 4047               | 7/12        | 33.6                      | 210                             | 1556                           | 7.4        | 504                            | 130   | *                             | 1850                           | 1420                                  | 430  | 1052                           |

\* Time of death not known. Calculations made using last hemoglobin determinations (column 8).

Column 6 = column 5 ÷ column 4.

Column 7 = column 4 × 2.4.

Columns 8 and 9. Values calculated using the normal as 100 per cent.

Column 9 = extrapolation to time of death.

Column 10 = column 3 × 0.055 × 1000 (Boycott and Damant, Journ. Physiol., 1907-8, xiv, 36).

Column 11 = column 10 ÷ column 9.

Column 12 = column 10 - column 11.

Column 13 = column 5 - column 7.

Examining the last two columns of the table it is evident that in only one instance the amount of extra fluid in the lung is less than the calculated loss of fluid from the blood. In some instances the extra fluid in the lung is much greater than that lost by the blood. Little or no water is drunk by goats in this condition and the volume of urine

excreted is small, so that the external factors do not confuse the picture. Even with the relatively large errors of calculation involved, the conclusion seems justified that the loss of fluid by the blood can be accounted for by the excess of liquid in the edematous lung.

The evidence suggests that the muscles, etc., do not imbibe water and cause the concentration of the blood. In fact it would appear that water may be drawn from some tissues to make up part of the volume of liquid in the lung. We are thus finally led back to our original point of view that the development of the edema of the lungs and the concentration of the blood are interrelated and are the important factors in the pathological condition studied. With this fact established it is justifiable to conclude that the development of the edema of the lungs is the primary factor in the condition and that the development of the edema causes the concentration of the blood.

#### SUMMARY

The continuous administration of oxygen to goats gassed with chlorpicrin did not inhibit the concentration of the blood.

The percentage saturation of the hemoglobin with oxygen was normal even after a considerable concentration of the blood had occurred.

The concentration of the blood is not caused by the imbibition of water by the tissues as the result of oxygen want.

The loss of water from the blood is therefore due to the development of the edema of the lungs.

We wish to thank Mr. J. Barcroft and the staff of the Physiological Laboratories of the R. E. Experimental Station, Porton, England, for the many kindnesses extended to us throughout the course of our investigations there.



# THE EFFECT OF ADRENALIN, DESICCATED THYROID AND CERTAIN INORGANIC SALTS ON CATALASE PRODUCTION

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Dahm and Steck (1) found that the ingestion of urea and of sodium chloride increased oxidation. This observation has been repeated and confirmed by Tangl (2) on curarized animals with their kidneys removed. Raeder (3) also found that saline injections especially if hypertonic increased the respiratory exchange. Loewy (4) observed that the ingestion of small amounts of water (100 cc.) produced no change in oxygen consumption, while Speck (5) found that drinking large quantities (1250 cc.) did. According to Lusk (6) water, sodium chloride and urea have no effect on the respiratory exchange. Magnus-Levy (7) observed an increased carbon dioxide output in a man fed thyroid extract and an increased oxygen intake in cases of exophthalmic goiter. It is recognized that tetrahydro- $\beta$ -naphthylamin increases oxidation in the body and decreases heat elimination. Grafe (8) has shown that the administration of ammonium carbonate, ammonium chloride and sodium carbonate increases oxidation.

We (9) had found that whatever increased oxidation in the body, the ingestion of food, for example, produced an increase in catalase by stimulating the alimentary glands, particularly the liver, to an increased output of this enzyme, and that whatever decreased oxidation, narcotics, for example, produced a decrease in catalase by direct destruction and by decreasing its output from the liver. The object of the present investigation was to determine if adrenalin, tetrahydro- $\beta$ -naphthylamin, desiccated thyroid, water, sodium chloride, ammonium chloride, sodium carbonate, ferric chloride, sodium citrate, ammonium carbonate, urea, triacetin and saccharin would or would not produce an increase in catalase. The amounts of the substances used will be given in the description of the individual experiments. The animals used were dogs and rabbits. After etherizing these animals

and opening the abdominal wall, each of the substances was introduced into the upper part of the intestine. The catalase in 0.5 cc. of blood was determined before, as well as at fixed intervals after the introduction of the materials. The determinations were made by adding 0.5 cc. of blood to hydrogen peroxide in a bottle at approximately 22°C. and the amount of gas liberated in 10 minutes was taken as a measure of the catalase content of the 0.5 cc. of blood.

In figure 1 are shown the effects of the introduction into the intestines of rabbits of urea, sodium chloride, water, triacetin and glycerine. The amounts of the substances used are indicated on the chart. Seventy-five cubic centimeters of water were used in dissolving the different substances. The figures along the ordinate (0 to 780) indicate amounts of catalase measured in cubic centimeters of oxygen and those along the abscissae time in minutes.

It may be seen that 2 grams of urea, 1 gram of sodium chloride and 15 cc. of water per kilo produced no increase in catalase in keeping with Lusk's observation that amounts of these substances as small as these produced no increase in oxidation, while 10 grams of urea, 10 grams of sodium chloride and 150 cc. of water per kilo did produce an increase in catalase in keeping with the observations of Dahm and Steck, Tangl, Speck and Raeder, that large amounts of these substances produce an increase in oxidation.

It may also be seen that triacetin is more effective in producing an increase in catalase than glycerine, this result being attributed to the presence of the acetic acid radicle in the glycerine molecule.

The second part of the paper is concerned with determining the mode of action of the substances already mentioned as well as several other substances in producing an increase in catalase. The animals used were dogs and the method for determining catalase was the same as that already described. The substances were dissolved in 200 cc. of distilled water and were introduced at body temperature into the upper part of the small intestine. The catalase content of 0.5 cc. of blood taken from the liver, portal and jugular veins was determined before as well as at certain fixed intervals after the introduction of the materials. In figures 2, 3 and 4 the continuous line curves were constructed from data obtained from the blood of the liver, the discontinuous line curves from the blood of the portal, and the dotted line curves from that of the jugular vein.

In figure 2 it may be seen, as we have found before, that the introduction of glycocoll produced an increase in catalase as is indicated by

the increase in the amount of oxygen liberated by the blood. It may be seen further that this increase is greater, particularly during the first fifteen minutes, in the blood of the liver than in the blood of the jugular or portal veins.

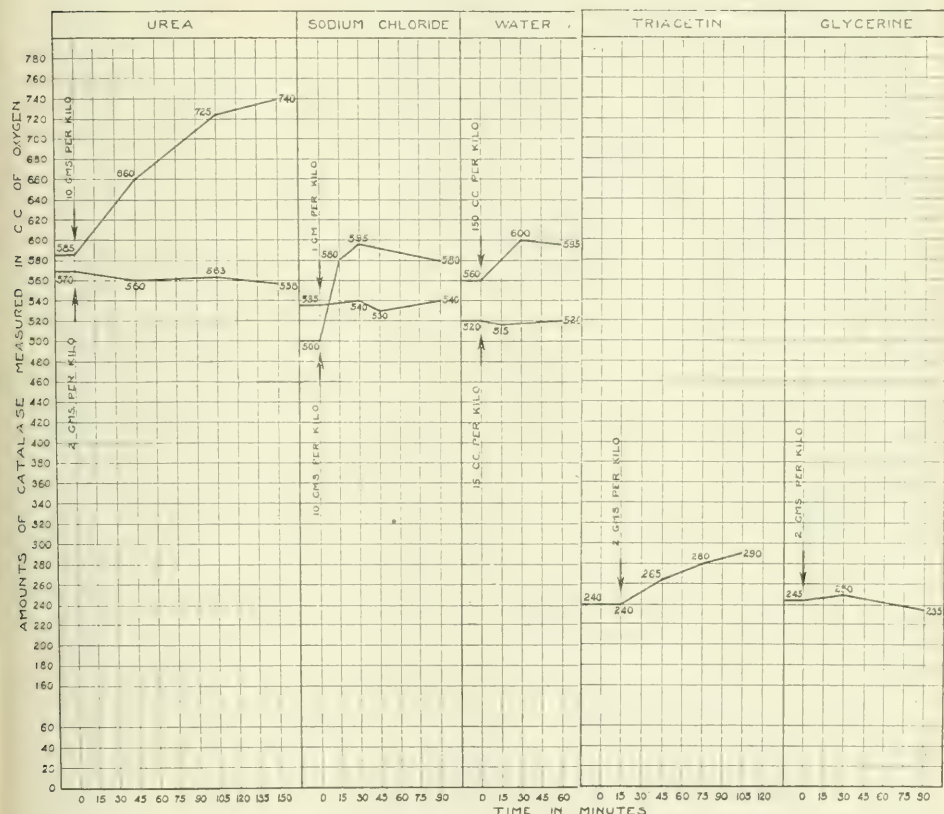


Fig. 1. The effect of the introduction into the intestines of rabbits of the substances named in the chart on blood catalase.

Under urea it may be seen as with glycocholate that this substance increases the catalase of the blood of the liver more rapidly than that of the portal and jugular veins. Under glycerine and triacetin it may be seen that while 5 grams per kilo of glycerine produced no increase in catalase, a similar amount of triacetin produced an increase thus showing that triacetin is more effective in this respect than

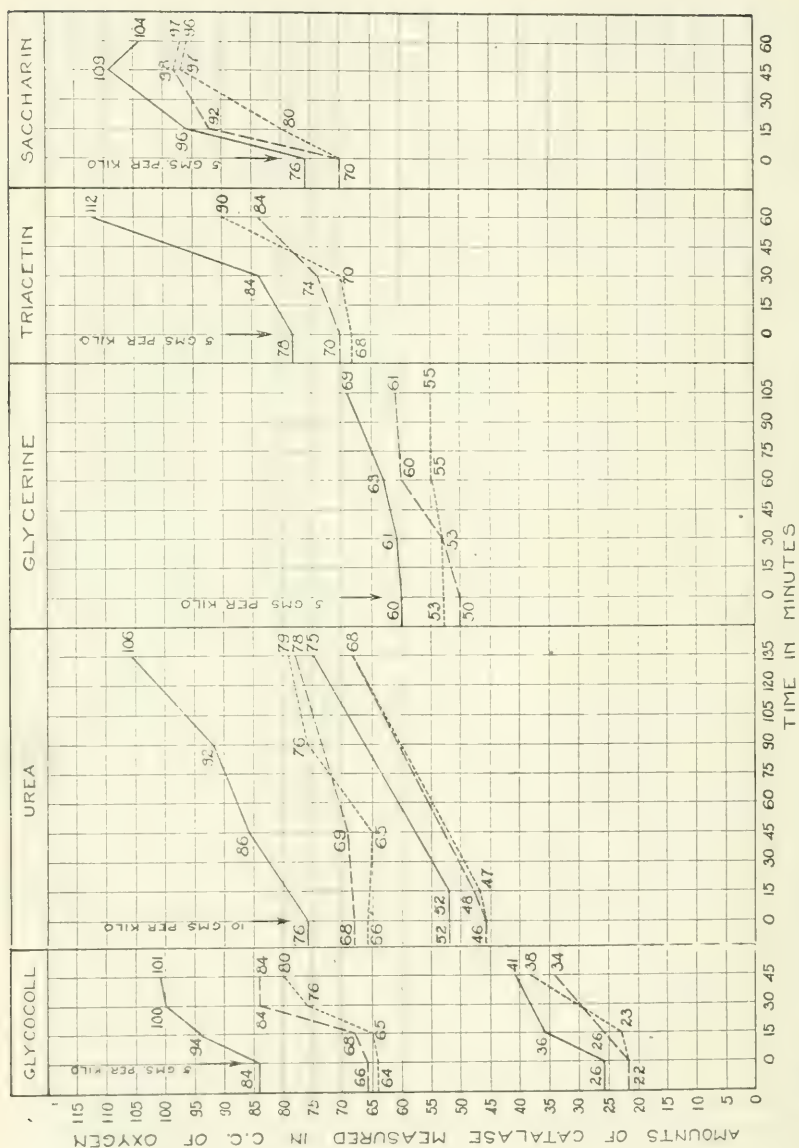


Fig. 2. The effect of the introduction into the intestines of dogs of the substances named in the chart on blood catalase. The continuous line curves were constructed from data obtained from the blood of the liver; the discontinuous ones from the blood of the portal and the dotted line curves from the blood of the jugular vein.



glycerine. It may also be seen that 5 grams per kilo of saccharin produced an increase in catalase. It (10) had been found that 5 grams of sugar would not produce so large an increase in catalase as is here shown to be produced by this amount of saccharin, hence so far as

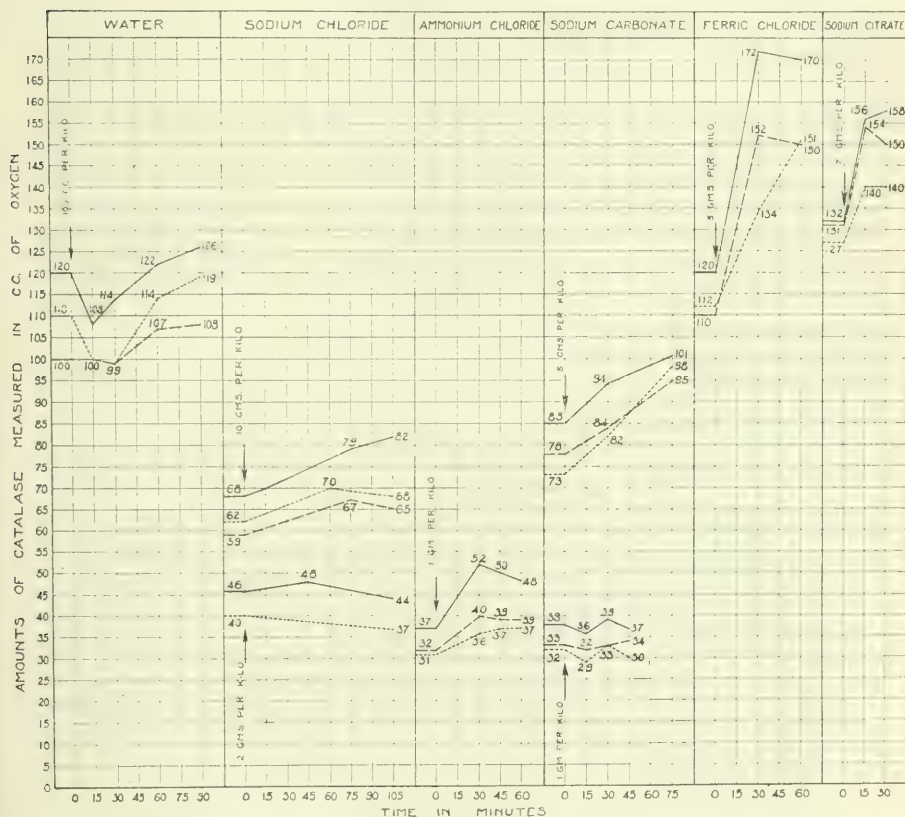


Fig. 3. The effect of the introduction into the intestines of dogs of the substances named in the chart on blood catalase. The continuous line curves were constructed from data obtained from the blood of the liver; the discontinuous ones from the blood of the portal and the dotted line curves from the blood of the jugular vein.

catalase production is concerned saccharin is more effective than sugar. It would seem that in addition to being a sweetening agent, saccharin, although not oxidized itself to give rise to energy, as is the case with sugar, serves to stimulate the alimentary glands to an in-

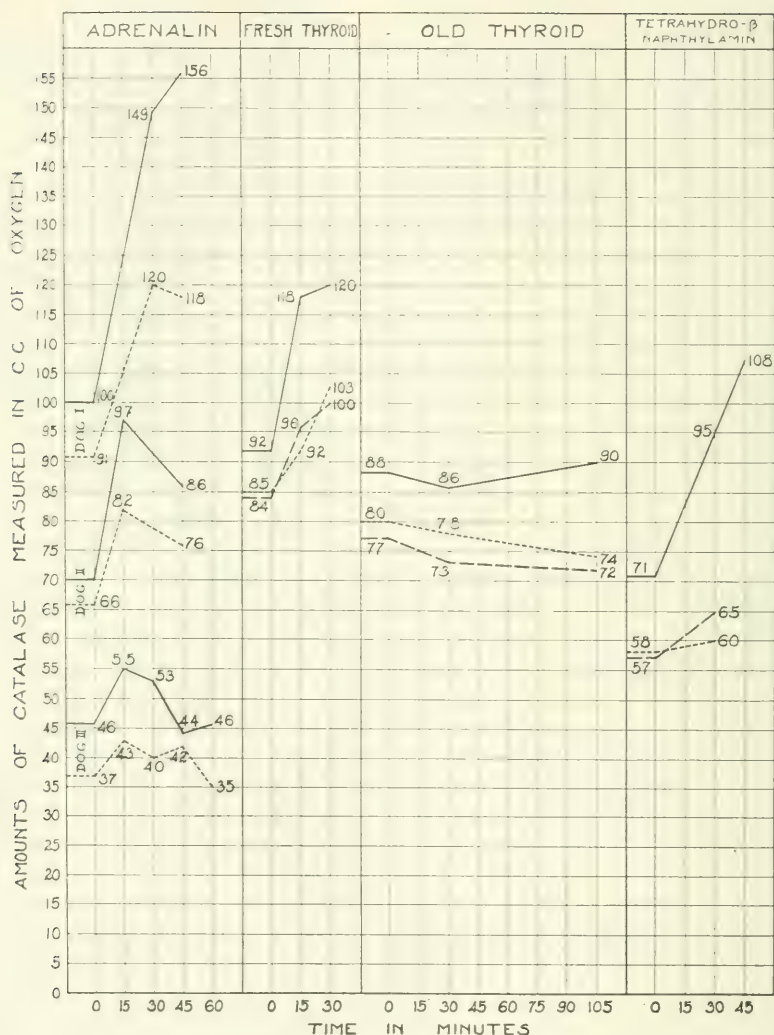


Fig. 4. The effect of the introduction into the intestines of dogs of the substances named in the chart on blood catalase. The continuous line curves were constructed from data obtained from the blood of the liver; the discontinuous ones from the blood of the portal and the dotted line curves from the blood of the jugular vein.

creased output of catalase thus facilitating the oxidation of the other food materials.

In figure 3 it may be seen that the introduction of water, sodium chloride, ammonium chloride, sodium carbonate, ferric chloride and sodium citrate into the alimentary tract of dogs produced an increase in the catalase of the blood and that this increase was brought about by stimulating the alimentary glands, particularly the liver, to an increased output of this enzyme. The fact that these salts produce an increase in catalase and hence in oxidation may in some measure account for the beneficial effect of certain mineral waters which contain these substances in conspicuous amounts.

In figure 4 is shown the effect of adrenalin, freshly prepared and old thyroid and tetrahydro- $\beta$ -naphthylamin on catalase production. Three cubic centimeters of a 1:1000 solution of adrenalin chloride were introduced into the portal vein in dog 3 and 5 cc. in dog 2. In these two dogs the solutions were injected as quickly as could be conveniently done while in dog 1, 10 cc. of a 1:1000 adrenalin chloride solution diluted to 50 cc. were injected at a rate of about 1 cc. per minute, thus requiring approximately 30 minutes for the injection. It may be seen that the adrenalin increased the catalase of the blood by stimulating the liver to an increased output of this enzyme.

As a result of the work of Blum (11), Vosburgh and Richards (12), Dreyer (13), Oliver and Schaefer (14), Cannon and de la Paz (15) it is now believed that during combat the adrenals are stimulated to an increased output of adrenalin and that this produces a constriction of the small blood vessels of the abdominal viscera, thus increasing the blood supply to the heart, skeletal muscles and nervous system; that it hastens the coagulation of the blood and increases the output of sugar from the liver. It is evident that the result of diverting the blood from the abdominal viscera into the heart, skeletal muscles and nervous system during combat is to render conditions more favorable for the increased action of these organs; that the hastening of the coagulation is to stop more quickly the bleeding from any superficial wound that may be inflicted, and that the flushing of the blood with sugar is to insure a plentiful supply of oxidizable material to the muscles. While the preceding hypothesis explains certain phases of adaptation of the organism for combat, it does not explain how the increased oxidation is brought about which gives rise to the energy for the fight. We had already found that the stimulation of the splanchnic nerves to the liver produced an increased output of catalase

from this organ, and had suggested that the increased oxidation during combat might be due to this increase in catalase. The fact that adrenalin stimulates the liver to an increased output of catalase suggests that this result may occur during combat when there is an increased output of adrenalin into the blood.

It may be seen in the figure that the introduction into the alimentary tract of freshly prepared thyroid produced an increase in catalase while old thyroid did not. The old thyroid was a Parke-Davis preparation which had been standing in the laboratory for four years, while the fresh thyroid was material recently purchased from this same company. The old material was fed to cats and found to have lost its virtue while the new material produced the characteristic effects, loss of weight, etc. The amount of the thyroid introduced into the intestines of the dogs was 1 gram per kilo dissolved in 200 cc. of water.

We had already found that the feeding of thyroid to cats increased very greatly the catalase of the blood. The experiments described in this paper on the introduction of thyroid into the alimentary tract of dogs suggest that the increase in the catalase of the blood of animals fed with thyroid is due to the stimulation of the liver to an increased output of this enzyme. Winternitz (16) found that "the removal of the thyroid gland caused a drop in the catalase activity of the blood which was compensated if thyroid were fed" and that in hyperthyreosis the catalase of the blood tends to increase while in hypothyreosis it assumes a lower level than normal. Becht (17), on the other hand, claims that thyroid feeding decreases the catalase of the blood. It should be mentioned also in this connection that Becht holds that narcotics slightly increase the catalase content of the blood, while we found that they produce a great decrease both in vivo and in vitro.<sup>1</sup>

Under tetrahydro- $\beta$ -naphthylamin it may be seen that the introduction into the intestine of 0.8 gram per kilo of this substance dissolved in 200 cc. of water stimulated the liver very greatly to an increased output of catalase which is offered in explanation of the increased oxidation produced by this substance.

<sup>1</sup> Owing to our proximity it has been suggested and even urged that Doctor Becht and myself carry out some joint experiments in an attempt to clear up the differences in our results. I am sorry to say that Doctor Becht seems to be unwilling to carry out such experiments.



## SUMMARY

The introduction into the alimentary tract of relatively small amounts of water (15 cc.), of sodium chloride (1 gm.), and of urea (2 gms.) per kilo, produces no increase in catalase in keeping with Lusk's observation that small amounts of these substances produce no increase in oxidation, while the introduction of large amounts of these substances, 1500 cc. of water, 10 grams of urea per kilo and 10 grams of sodium chloride per kilo do produce an increase in catalase in keeping with the observations of Dahm and Steck, Tangl, Speck and Raeder, that large amounts of these substances produce an increase in oxidation.

The injection of adrenalin into the portal vein stimulates the liver to an increased output of catalase. This fact suggests that the increased amount of adrenalin thrown into the circulation during combat may stimulate the liver to an increased output of catalase, and in this way aid in bringing about the increase in oxidation occurring during combat.

Desiccated thyroid when introduced into the alimentary tract stimulates the liver to an increased output of catalase. This observation suggests that the increase in the catalase of the blood which may be responsible for the increase in the respiratory exchange of an animal when fed with thyroid or in exophthalmic goiter is probably due to the stimulation of the liver to an increased output of catalase.

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## A NOTE ON THE QUESTION OF THE SECRETORY FUNCTION OF THE SYMPATHETIC INNERVATION TO THE THYROID GLAND

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Considerable interest has attached itself to the question of the innervation of the thyroid gland, and as yet the conflicting evidence presented by different experimenters does not permit the drawing of definite conclusions. Asher and Flack (1), by stimulating the laryngeal fibers from the vagus, found the blood pressure response to adrenalin injection to be greatly increased. Asher (2) later decided that this increased effectiveness of adrenalin was due to the sensitizing action of the thyroid secretion on sympathetic endings following stimulation of the nerve fibers to the gland. Cannon and Cattell (3) demonstrated an electrical variation in the gland, which they interpreted as evidence of secretory activity following stimulation of the cervical sympathetic fibers, while stimulation of laryngeal fibers from the vagus was ineffective. Levy (4) observed an increased blood pressure response to adrenalin after stimulation of cervical sympathetic and after adrenalin injection, which he accepted as evidence of sensitization of sympathetic endings by the thyroid secretion following stimulation. Cannon, Ringer and Fitz (5), by fusion of the central end of the phrenic nerve to the peripheral portion of the cut cervical sympathetic trunk, obtained symptoms of sympathetic stimulation in the eye and in the ear vessels of rabbits, and also symptoms similar to those of hyperthyroidism, such as rapid pulse, increased metabolism and hyperexcitability. These latter symptoms disappeared on removal of the thyroid on the side of the nerve suture, so the evidence was taken to indicate thyroid stimulation by the phrenic impulses.

On the other hand Troell (6) failed to obtain any evidence of thyroid stimulation, either symptomatically or microscopically, on suturing the phrenic to the cervical sympathetic. Burget (7), and Marine,

Rogoff and Stewart (8) also reported negative results by this same method. Manley and Marine (9) and others, have reported that thyroid transplants survive and function in various parts of the body, regardless of nerve supply, so that secretory nerves to the gland are at least not essential to its activity.

From the above contradictory evidence presented it is evident that any new contribution should be welcome. The object of the experiments described in this paper was to determine whether the thyroid could be stimulated to greater activity through repeated injections of cocaine, the basis of the use of cocaine being the work of Froelich and Loewi (10), in which they demonstrate quite conclusively that cocaine sensitizes sympathetic nerve endings to the action of adrenalin and to sympathetic stimulation.

#### EXPERIMENTAL

The subcutaneous injection of 35 to 50 mgm. cocaine hydrochloride per kilo body weight, or of 10 mgm. cocaine hydrochloride accompanied by .1 mgm. adrenalin per kilo body weight, calls forth in the rabbit symptoms of undoubted sympathetic stimulation, such as maximal dilatation of the pupils, slight exophthalmos, constriction of the ear vessels leaving the ears cold to the touch, relaxation and loss of tone in the intestines, and erection of the hair over the body. That it is either a sensitization of the sympathetic endings to the normal flow of impulses over these nerves, or else an increased central stimulation, can be shown by cutting one sympathetic nerve in the neck and then noting the effect of cocaine on the pupils and ear vessels. Effects of the drug were seen only on the side having the intact nerve supply. Further, on frightening the animal, as by dropping from a distance of a foot or so onto the observation table, a slight dilatation of the pupil on the side of the intact nerve occurs within one-half second and disappears within one second after cessation of the frightening, thus indicating that the results obtained are not due to a sudden outpouring of epinephrin, but far more likely due to impulses traversing the sympathetic nerves.

Having established to my satisfaction, then, that cocaine acts in the normal rabbit by increasing the effectiveness of the normally occurring sympathetic impulses, I next tried to determine the effects on the thyroid gland as evidenced by histological changes in its structure. Specimens of the gland were removed aseptically before and after the cocaine treatment and examined for changes in their microscopic

appearance. Ordinary aseptic precautions were observed in the injections. Since all references in the literature indicate that cocaine is only slowly eliminated, (Wiechowski (11), Grode (12)) the treatment was begun with daily injections of sufficient strength to produce a maximal mydriasis of the pupils lasting about 30 minutes, that is, about 10 mgm. of cocaine per kilo body weight. However, since the effects seemed to be over within an hour, the injections were increased to 3 or 4 a day. In one series of five rabbits thus treated for 8 days there was observed no change whatever in the microscopic appearance of the thyroid glands. A second series of three rabbits was run, each animal receiving 5 to 10 injections a day for 11 days, and again no changes in the glands were observed. Neither did the rate of growth or general appearance and behavior of the animals indicate any lasting results from the use of the drug.

Since the strength or effectiveness of the impulses over the sympathetic fibers should have been greatly augmented by the continued use of cocaine producing symptoms of thyroid hyperactivity and morphological changes in the gland, the evidence from these experiments contributes to the indication of a lack of secretory function of the sympathetic fibers to the thyroid gland.

*Note.* The experimental part of this work was carried on in the Laboratory of Pharmacology of the University of Chicago at the suggestion, and under the direction, of Dr. A. L. Tatum, for whose aid and suggestions I desire to express my sincere thanks.

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## THE HYPERGLYCEMIA-PROVOKING ABILITY OF ASPHYXIAL BLOOD

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The rôle of adrenalin in the asphyxial hyperglycemia and glycosuria has been a question for a long time and is not yet decided.

Pollak (1), in classifying the various experimental conditions in which hyperglycemia occurs, regarded asphyxial hyperglycemia as due to a stimulation of the sympathetic nervous system akin to that produced by the injection of adrenalin. The experimental basis for this opinion lay in the fact that the asphyxial glycosuria and hyperglycemia could not be prevented by resecting the splanchnic nerves of both sides, though they were remarkably lowered in degree by this operation.

Emil Starkenstein (2) carried out a number of experiments in which he proved histologically a diminution of the chromaffin substance in the medullary cells of the adrenals of asphyxiated rabbits. Extracts from the adrenals of these asphyxiated rabbits also exhibited a lessened power to raise the blood pressure when injected into normal rabbits.

The histological staining abnormalities as well as the decrease of the blood-pressure-raising substance could be prevented by cutting the splanchnic nerves before suffocation. He presumed upon this experimental ground that the asphyxial stimulation acts first upon the blood-sugar-controlling centre of the brain as in the case of Piquère, and then this stimulation is transmitted along the sympathetic nerve to the suprarenal, causing a hypersecretion of suprarenin by exciting the medullary cells and this adrenalin, transferred into the circulation, gives rise to the increased sugar content of the blood.

Czubalski (3), supporting the view that hypersecretion of adrenalin occurs in asphyxia, proved that the rise of the blood pressure of animals does not occur if previous to suffocation the adrenals are removed. Borberg, Frederica and many others carried out similar experiments to prove the rôle of the suprarenals or suprarenin in asphyxial glycosuria and hyperglycemia. But most of the methods of investigation employed by these authors were indirect and did not permit a definite decision as to the rôle of suprarenin.

In order to solve the question whether the adrenals are involved in asphyxial hyperglycemia, it seems to be the wisest method to study this hyperglycemia in animals whose adrenals were removed entirely. This is, however, not an easy task for most animals live only a short time after the operation. It would not be safe, as Stewart has pointed out, to take the experimental results obtained from animals in such a condition that they are about to die, as evidence of an important rôle of adrenalin in experimental diabetes. The only animal which can survive double adrenalectomy for long without any pathological symptoms, is the rabbit. Consequently most of the experiments in this field have been performed upon this animal. Many investigators have stated that hyperglycemia or glycosuria do not occur after removal of the adrenals.

The non-occurrence of Piquè glycosuria after adrenalectomy was proved by Meyer (4), that of the glycosuria provoked by splanchnic stimulation was proved by Thomas (5) and Macleod (6), that of the CO-glycosuria was proved by Starkenstein. Kahn, Starkenstein and others are said to have proved that various kinds of glycosuria do not manifest themselves in rabbits even one year after adrenalectomy (7). Macleod and Pearce (8) obtained similar results in regard to hyperglycemia.

Kahn has reported in his more recent paper the non-occurrence of hyperglycemia following diabetic puncture, CO, diuretin, emotion and salt, in rabbits after successful adrenalectomy (9).

These results obtained by various authors might be considered as sufficient evidence to prove the indispensable intervention of the adrenals or of adrenalin in all experimental diabetes and hyperglycemias, except the renal and pancreas diabetes, if other authors had not obtained the opposite results. Unfortunately many exceptional cases have been observed, in which adrenalectomy of both sides did not prevent the experimental glycosuria or hyperglycemia, especially in other animals than rabbits. For instance, Wertheimer and Battez (10)

could obtain glycosuria in cats by Piqûre after adrenalectomy. Starkenstein observed a remarkable glycosuria produced by stimulating the splanchnic nerves for a long time in rabbits deprived of their adrenals.

Kahn comments upon the occasional occurrences of the glycosuria or hyperglycemia after adrenalectomy in the following words:

Es wird also anzunehmen sein dass die auf dem direkten Wege erzeugte Reizung der sympathischen Nervenende in der Leber, weder bei der CO noch bei Piqûre hoch gradig genug sei, um zur plötzlichen ueberstürzten Glykogen Mobilisierung zu rühren. Diese notwendige Erregungsgrösse wird erst durch die Addition von Adrenalin zu Wege gebracht. Wenn aber durch irgend eine unbekannte Ursache, die Erregungsgrösse erbracht im direkten Wege gross genug sei, dann treten die Glykosurie oder Hyperglykaemie in nebennierenlosen oder Splanchnicus resezierten Tieren auf.

There are too many presumptions which have not sufficient experimental foundation in the explanation offered by Kahn. But, in any case, this much seems to be true, that various experimental hyperglycemias can be made difficult to manifest themselves by extirpation of adrenals even in the presence of a sufficient deposit of liver glycogen. Besides there is one more possibility which may account for the hyperglycemia after extirpation of the suprarenals, in favor of the adrenalin theory, and that is the probability of adrenalin secretion from other glands than the adrenals. The carotid glands, Zuckermandl's glands, Luschka's glands and others which contain chromaffin cells may produce and secrete suprarenin. The amount of suprarenin produced from this source may be very small under normal conditions of life. But it is quite conceivable that this amount may rise compensatorily when the main sources of adrenalin are removed and extraordinary stimulations are applied to the secretory nerves.

Recent attempts to elucidate this problem have been made by two American investigators and their students, namely, Cannon and Stewart. They tried to determine the increase of adrenalin in the blood of animals under experimental conditions directly by means of segments of uterus and intestine. And curious to say, their results were just opposite to each other, Cannon and his students having obtained positive (11), Stewart and his collaborators having obtained negative results (12).

Stewart worked on cats in which one adrenal was removed, and the nerve supply for the other was cut off. It is not surprising that he was able to provoke asphyxial or other experimental hyperglycemia in such animals because, as was cited before, the experimental hyper-



glycemia manifests itself sometimes even in cats deprived of both adrenals. But his negative results, in attempting to detect the epinephrin output from the remaining adrenal, upon which experimental basis he infers that the asphyxial hyperglycemia occurs without any intervention of adrenalin, may have been due to the exhaustion of epinephrin, because the methods employed by Stewart to obtain the blood sample included numerous procedures which have been shown by various investigators to cause experimental hyperglycemia. The fixation of animals upon the operation table, narcotization, laparotomy, ligation of a large artery and vein, etc., have all been reported as causing a rise in blood sugar. And all of these hyperglycemias may be accompanied by hypersecretion of adrenalin, as many authors believe. Even if the asphyxial stimulation leads to hypersecretion from the adrenal glands, this secretory stimulation can not be very strong. The various manipulations involved in obtaining the asphyxial blood sample may very probably, therefore, have exhausted the adrenals to such a degree that no more epinephrin could be elicited from the glands by such a weak stimulation as asphyxia. Hypersecretion under these conditions might only have been expected in response to such an extraordinary stimulation as the direct massage of the glands or strong galvanization of the secretory nerve, which Stewart found to cause an increased adrenalin output. Indeed the entire lower part of the animal body with many abdominal organs was already in an asphyxial condition at the conclusion of the preparations for securing the blood sample, if I am not mistaken. And again, as stated above, the epinephrin may be produced and discharged into the circulation by other glands than the adrenals.

There is too great a discrepancy between Stewart's results and those of Cannon, who has proved a remarkable increase of adrenalin in the caval blood and sometimes even in the heart blood during asphyxia. Whether this discrepancy is due to the inaccuracy of the methods employed for the estimation of epinephrin or due to the diversity of methods of obtaining the blood sample, is a question which calls for further investigation.

The results of the experiments which I have to report here seem to be rather in accordance with the adrenalin theory.

I have undertaken this work with the object of ascertaining whether the asphyxial blood has any appreciable ability of provoking hyperglycemia when transferred into the circulation of other animals and have obtained a positive result.



Lepine and Boulud (13) have proved the existence of a substance in asphyxial blood which can induce glycosuria when injected into animals. Lepine called this substance "Leucomaines diabétogènes." He extracted it from the asphyxial blood of dogs, and could cause a remarkable glycosuria in guinea pigs by injecting this extract. According to his hypothesis, this substance is produced in the blood by the lack of oxygen, and causes the glycosuria by preventing the oxidation of sugar. But the Leucomaines diabétogènes is said to be capable of inducing a glycosuria lasting two to three days by only one subcutaneous injection. The asphyxial glycosuria and hyperglycemia do not last so long generally. They usually disappear within five or six hours after the removal of the cause. Only the suffocation with CO-gas is reported to be sometimes followed by glycosuria lasting twenty or thirty hours, but this is probably due to the CO poisoning and not to the asphyxia itself. Hence it is very doubtful whether this substance which Lepine separated from asphyxial blood has anything to do with asphyxial glycosuria. Besides no subsequent investigators have been able to confirm the existence of this substance.

In my experiments rabbits were used as experimental animals. The blood was drawn by heart puncture from rabbits during deep asphyxiation and was injected into the auricular vein of other normal rabbits and then the blood sugar of the injected animals was determined twenty to thirty minutes after the injection.

#### DESCRIPTION OF TECHNIQUE

The hydroöxylamin method of Momose (14) was used for the determination of blood sugar in the beginning of the work, but in the latter part I was obliged to use some other method because I could not obtain the apparatus for Momose's method. I have chosen, therefore, Benedict's method (15) in place of Momose's.

The principle of Momose's method is to let a measured amount of a given sugar solution react with a boiling copper sulphate solution of a known amount larger than the amount of sugar to be reduced, in a atmosphere of ammonia gas in order to avoid the intervention of atmospheric oxygen and then to titrate the amount of copper left unreduced, by means of a standard solution of hydroöxylamine sulphate. The amount of sugar can then be calculated from the amount of the hydroöxylamine solution used. This method gives a very fair result, though it is a little complicated and needs some training in practice. The reason

for employing hydroöxylamin, is that the reducing power of sugar in an alkaline solution is not strong and it decreases with the decreasing concentration of the metal salt to be reduced. If, therefore, the sugar solution were titrated up to the terminal reaction as in the Pavy's method, we can not get a correct value, but a somewhat larger value is obtained. Hence, in this method, the terminal reaction is determined with hydroöxylamine solution which has a strong reducing power. The blood protein is precipitated by colloidal iron. Momose obtained 0.1383 for the physiological percentage of blood sugar of rabbits. But I got a little lower value, probably owing to the precautions I took in obtaining the blood sample. I chose old rabbits and drew the blood from the auricular vein avoiding agitation of the animals and did not draw more than 6 cc. at one time from one rabbit. The blood was taken uniformly 3 hours after the morning feeding. Some of the results are given below.

| RABBIT NUMBER | DATE OF THE DETERMINATION | PERCENTAGE OF SUGAR |
|---------------|---------------------------|---------------------|
| N. 1          | February 9                | 0.0960              |
| N. 2          | February 9                | 0.1036              |
| N. 3          | February 9                | 0.1292              |
| N. 4          | February 11               | 0.1264              |
| N. 5          | February 11               | 0.1132              |
| N. 1          | February 13               | 0.1279              |
| N. 4          | February 14               | 0.1008              |
| N. 3          | February 14               | 0.1270              |
| N. 2          | February 14               | 0.1124              |

*Experiment 1.* Healthy rabbits were suffocated by pressing the trachea with fingers, taking precautions not to press the carotids and vagus, and as soon as the animals were unconscious the pressing fingers were released and artificial respiration was applied. When the animals had become conscious, once more asphyxiation was brought about in the same way as before, and during this second asphyxia the blood was drawn by heart puncture with a sterile hypodermic needle, into about 0.01 gram of hirudin. (I must offer my heartiest thanks to Professor Tatum of Chicago University for his kindness in giving me the hirudin.)

Asphyxial blood coagulates quite slowly, but it is liable to coagulate within the needle when we wish to reinject the blood into other animals unless an anticoagulant is used.

The asphyxial blood thus taken was introduced immediately into the auricular vein of normal rabbits in which the percentage of blood sugar

had been previously determined. A small portion of the asphyxial blood was left for the determination of sugar.

The blood was drawn 20 to 30 minutes after the injection of the asphyxial blood from the auricular vein of the injected animals and its percentage of sugar was estimated. In case the rabbits did not revive from the first suffocation, the asphyxial blood drawn from the dead animals was used. Many rabbits died when the blood was taken during the second asphyxiation.

The results thus obtained are given below.

| NUM-<br>BER | WEIGHT<br>OF THE<br>INJECTED<br>RABBITS | SUGAR<br>BEFORE<br>INJECTION | AMOUNT<br>OF THE<br>INJECTED<br>BLOOD | SUGAR OF<br>THE<br>INJECTED<br>BLOOD | FATE OF THE SUFFOCATED RABBITS | SUGAR<br>AFTER<br>INJE-<br>TION |
|-------------|---|------------------------------|---------------------------------------|--------------------------------------|--------------------------------|---------------------------------|
|             | <i>grams</i>                            | <i>per cent</i>              | <i>cc.</i>                            | <i>per cent</i>                      |                                | <i>per cent</i>                 |
| 1           | 2905                                    | 0.121                        | 11.5                                  | 0.266                                | Alive                          | 0.135                           |
| 2           | 3202                                    | 0.090                        | 14.0                                  | 0.146                                | Alive                          | 0.134                           |
| 3           | 2650                                    | 0.147                        | 12.0                                  | 0.279                                | Alive                          | 0.148                           |
| 4           | 2895                                    | 0.128                        | 17.0                                  | 0.320                                | Died in second asphyxiation    | 0.149                           |
| 5           | 3430                                    | 0.115                        | 10.0                                  | 0.208                                | Alive                          | 0.152                           |
| 6           | 2250                                    | 0.105                        | 15.0                                  | 0.217                                | Alive                          | 0.178                           |
| 7           | 2280                                    | 0.123                        | 15.0                                  | 0.265                                | Died in second asphyxiation    | 0.197                           |
| 8           | 2545                                    | 0.136                        | 15.0                                  | 0.305                                | Died in first asphyxiation     | 0.141                           |
| 9           | 3160                                    | 0.105                        | 18.0                                  | 0.196                                | Alive                          | 0.158                           |
| 10          | 2750                                    | 0.122                        | 17.0                                  | 0.190                                | Died in first asphyxiation     | 0.204                           |
| 11          | 2455                                    | 0.120                        | 19.0                                  | 0.283                                | Died in second asphyxiation    | 0.172                           |

All animals used in the experiment were tested previously for the existence of isolsyn in their blood because I feared that the hemolysis caused by isolsyn might induce a kind of partial internal asphyxiation by destroying the red blood corpuscles of the injected animals, which in turn might provoke hyperglycemia, just in the same way as hyperglycemia is believed to arise in CO-poisoning. In order to test for isolsyn, a small amount of the blood sample was drawn from the rabbits to be suffocated, the serum was separated and was mixed with the blood corpuscles taken from rabbits to be transfused, kept at 37°C. for one hour, and the result observed.

As will be seen in the table, the blood sugar percentage of rabbits injected with the asphyxial blood, showed an increase almost invariably twenty to thirty minutes after the injection. In no. 10. the increase was very remarkable. In this case the injected asphyxial blood had only 0.190 per cent sugar while the sugar percentage of the injected animal rose from 0.122 to 0.204. This might be considered as an error.

On the other hand it may be perhaps due to the greater sensibility of the injected animal than the suffocated for the substance causing disturbance of the sugar metabolism.

Before concluding that this ability of provoking hyperglycemia is a special property of asphyxial blood, it is necessary to ascertain the influence of normal blood upon the sugar metabolism when introduced into the circulation.

There are not many reports concerning the relationship between the sugar metabolism and the injection of protein. Henderson and Underhill (16) reported that hyperglycemia is caused by peptone injection owing to the acapnia induced by peptone poisoning. Hugh MacGuigan (17) found, on the contrary, hypoglycemia in peptone poisoning and generally in anaphylaxis. He writes in his report that "in making blood transfusion from one animal to another, they have noticed there is a general tendency for the blood sugar of the recipient to fall." But unfortunately he does not furnish the experimental basis for this conclusion and I was therefore uncertain as to the dose of blood transfused and the manner of transfusion and whether his transfusion was iso- or heterotransfusion. It was necessary, accordingly, to undertake the next experiment as control for the experiment already described.

**Experiment 2.** Blood was drawn from the auricular vein of healthy rabbits very cautiously without causing their agitation. Five to six cubic centimeters of normal blood were obtained from one animal. About 0.01 gram of hirudin was added to each 10 cc. of the blood which was then injected into the auricular vein of other rabbits, in which the blood sugar percentage had been previously determined. The injected animals received, therefore, the normal blood drawn from 2 or 3 other rabbits. The so-called *aderlass* hyperglycemia can not occur by drawing 5 or 6 cc. blood from one animal (18, 19).

*Control experiment with normal blood*

| NUMBER | WEIGHT OF THE<br>INJECTED RABBITS | SUGAR BEFORE<br>INJECTION | AMOUNT OF THE<br>INJECTED BLOOD | SUGAR 20 TO 30<br>MINUTES AFTER<br>INJECTION |
|--------|-----------------------------------|---------------------------|---------------------------------|--|
|        | <i>grams</i>                      | <i>per cent</i>           | <i>cc.</i>                      | <i>per cent</i>                              |
| 1      | 2795                              | 0.112                     | 15                              | 0.112  |
| 2      | 3110                              | 0.125                     | 15                              | 0.137  |
| 3      | 2560                              | 0.122                     | 15                              | 0.137  |
| 4      | 2785                              | 0.098                     | 15                              | 0.124  |
| 5      | 3310                              | 0.102                     | 15                              | 0.090  |
| 6      | 2845                              | 0.124                     | 18                              | 0.115  |
| 7      | 2405                              | 0.135                     | 18                              | 0.135  |
| 8      | 2600                              | 0.122                     | 18                              | 0.104  |



As will be seen from the table, I could not observe any distinct change of the blood sugar percentage caused by transfusion of the normal blood within the limits of dosage which I used. Such small variations as occurred in no. 4 or no. 9 may be caused by a slight technical failure of determination or by an agitation of the animals which can not be prevented.

This experiment proves also that hirudin had no effect on blood sugar, at least within the dosage I employed.

Thus I believe it certain that the ability of provoking hyperglycemia is a special property of asphyxial blood.

It is a question as to what agent of asphyxial blood this property is due. I carried out a few experiments endeavoring to find out the probable agent. But I could not reach any definite conclusion. In the next experiment I have shown that the excess of sugar contained in the asphyxial blood is not responsible for this ability.

*Experiment 3.* The injected asphyxial blood had somewhat large amount of sugar as shown in the table, some had 0.305 per cent and some had 0.320 per cent. In 18 cc. of such a blood there will be about 0.035 gram more glucose than the normal amount of sugar in that volume of blood, assuming the physiological percentage of sugar to be 0.12 per cent. If a rabbit is supposed to have 100 cc. blood (weight: blood = 20 : 1), and 0.035 gram sugar was introduced into circulation, this will make 0.035 per cent and the total percentage of the blood sugar of animals injected with the asphyxial blood, therefore, ought to have been 0.155 per cent at most, if the excess of sugar in the asphyxial blood were the sole cause of the rise of sugar percentage in the injected animals. But such calculation as this was not at all applicable as a matter of fact. The increase of sugar found never corresponded to the amount contained in the asphyxial blood. This fact alone is obvious evidence that the hyperglycemia is caused by some other agent than the excess of sugar. But further evidence is advanced in experiment 3 in order to fully eliminate the possibility that the excess of sugar may have disturbed the sugar metabolism of the injected animals.

Normal blood was drawn in the same way as described in the experiment 2, and 0.02 gram glucose was added to each 10 cc. of the blood, which was then injected slowly into the auricular vein of healthy rabbits. If the excess of sugar in the injected blood were the main cause of the hyperglycemia obtained in experiment 1, this normal blood with added glucose should give a similar result. The sugar determination was done with blood taken 20 to 30 minutes after the injection.

The results are as follows:

| WEIGHT OF RABBITS | SUGAR BEFORE INJECTION | AMOUNT OF INJECTED BLOOD | SUGAR AFTER INJECTION |
|-------------------|------------------------|--------------------------|-----------------------|
| <i>grams</i>      | <i>per cent</i>        | <i>cc.</i>               | <i>per cent</i>       |
| 3120              | 0.106                  | 15                       | 0.124                 |
| 2875              | 0.130                  | 15                       | 0.135                 |
| 2640              | 0.111                  | 15                       | 0.111                 |
| 2800              | 0.128                  | 15                       | 0.142                 |
| 2550              | 0.122                  | 18                       | 0.112                 |
| 2925              | 0.137                  | 18                       | 0.133                 |

As is shown in the table the result was not the same as that obtained with the asphyxial blood. The sugar percentage after injection was almost the same as before. This result agrees well with that of the experiment done by Thanhauser (20), who proved that the normal blood sugar percentage is restored within 15 minutes after the injection of 550 cc. of 7 per cent grape sugar solution into the vena mediana cubiti of man. Kleiner carried out similar experiments with animals and proved that a great part of the injected dextrose is transferred to the tissues and changed in polysaccharides very quickly (21).

*Experiment 4.* It is a well-known fact that in various kinds of experimental as well as clinical diabetes, the acidity of blood increases. In the asphyxial blood the increase of acidity is partly due to the excess of  $\text{CO}_2$ , but it has been proved by Araki (22) that lactic acid, oxalic acid, etc., are also responsible for it. This fact has been confirmed by subsequent investigators.

On the other hand, it is a fact beyond doubt that weak acid has an accelerating influence upon the activity of diastase or glycogenolytic ferment (23). Arguing from these facts and supported by other experimental data, many authors believe that the cause of asphyxial glycosuria or hyperglycemia is the excess of  $\text{CO}_2$  in the blood (24) or the increase of acidity (25).

The following experiment was, therefore, performed to investigate whether the hyperglycemia, provoked by the injection of the asphyxial blood, is not attributable to the acidity.

According to my experiment, the H-ion concentration of the normal arterial blood, taken from the left ventricle of the rabbit's heart, and that of the venous blood from the auricular vein, when determined by the gas chain method of Michaelis (26), is as follows:

*Arterial blood drawn by heart puncture*

| ROOM TEMPERATURE  | MILLIVOLT OBTAINED<br>WITH<br>STANDARD SOLUTION | MILLIVOLT OBTAINED<br>WITH BLOOD | PH   |
|-------------------|---|----------------------------------|------|
| <i>degrees C.</i> |   |                                  |      |
| 18                | 514.8   | 677.0                            | 7.39 |
| 20                | 515.0   | 682.2                            | 7.46 |
| 20                | 516.0   | 680.0                            | 7.42 |
| 20                | 516.0   | 678.0                            | 7.40 |
| 19                | 515.5   | 686.0                            | 7.54 |

*Venous blood taken from auricular vein*

|    |       |       |      |
|----|-------|-------|------|
| 20 | 516.6 | 668.0 | 7.21 |
| 20 | 516.1 | 681.0 | 7.43 |
| 19 | 516.0 | 673.3 | 7.32 |
| 20 | 516.0 | 675.5 | 7.34 |
| 20 | 516.5 | 672.5 | 7.30 |

The H-ion concentration of the asphyxial blood obtained in the same way as described in the experiment 1 and determined by the gas chain method, is as follows:

| ROOM TEMPERATURE  | MILLIVOLT OBTAINED<br>WITH<br>STANDARD SOLUTION | MILLIVOLT OBTAINED<br>WITH BLOOD | PH   |
|-------------------|---|----------------------------------|------|
| <i>degrees C.</i> |   |                                  |      |
| 19                | 516.0   | 634.0                            | 6.66 |
| 20                | 516.0   | 634.5                            | 6.64 |
| 20                | 516.6   | 629.0                            | 6.54 |
| 20                | 516.5   | 641.0                            | 6.75 |
| 20                | 516.2   | 652.5                            | 6.94 |

As mentioned above, the PH of the asphyxial blood was 6.54 to 6.94 while that of the physiological blood was 7.54 to 7.39 (arterial), 7.21 to 7.43 (venous). This acidity may have been the cause of the hyperglycemia in the recipients in our experiment 1. I therefore neutralized the asphyxial blood with alkali carbonate solution, and repeated the experiment with this neutralized asphyxial blood, to investigate whether the hyperglycemia will fail to occur after neutralization of the blood.

In order to neutralize the blood, the H-ion concentration was first determined by the indicator method and then  $\text{Na}_2\text{CO}_3$  solution (10 per cent) was added to the saline solution used for the indicator method until the PH became 7.5 to 7.6, and then the amount necessary to be added to the asphyxial blood was calculated from the amount required.

The blood thus neutralized was injected into healthy rabbits. (I wish to express here my gratitude for the kindness of Doctor Tashiro of Chicago University for lending me the apparatus for the determination of H-ion concentration.)

The results thus obtained are given below:

| WEIGHT OF RABBITS | SUGAR BEFORE<br>INJECTION | AMOUNT OF INJECTED<br>BLOOD | SUGAR AFTER INJECTION |
|-------------------|---------------------------|-----------------------------|-----------------------|
| <i>grams</i>      | <i>per cent</i>           | <i>cc.</i>                  | <i>per cent</i>       |
| 2815              | 0.135                     | 15                          | 0.172                 |
| 2310              | 0.128                     | 18                          | 0.144                 |
| 2570              | 0.096                     | 15                          | 0.180                 |
| 2645              | 0.137                     | 15                          | 0.188                 |
| 3105              | 0.119                     | 15                          | 0.156                 |
| 2760              | 0.131                     | 15                          | 0.145                 |
| 2650              | 0.122                     | 15                          | 0.127                 |

As cited in the table, I observed that the tendency to promote hyperglycemia remains in the asphyxial blood even when the blood was neutralized. Evidently, therefore, the acidity of the asphyxial blood samples was not responsible for the effect induced.

#### DISCUSSION

It is shown in this work that asphyxial blood causes a rise in the sugar content of blood when introduced into the circulation of other animals. It can not be stated what products of asphyxia act as the primary cause of asphyxial hyperglycemia. The lack of oxygen may form a primary cause by leading to deficient oxidation of carbohydrates, as Claude Bernald, Dastre, Lepine, Terrey, Araki and others believe, or the excess of CO<sub>2</sub> and the increased acidity may be the primary cause, as Edie, Moore, Roaf, Macleod and others have suggested, or the blood sugar may increase owing to a decrease of the activity of tissue oxidase as Underhill thought, or the asphyxial glycosuria and hyperglycemia may be due to the emotional disturbance or the fear of death, as Bang and Stenström opine, because it is not possible to suffocate animals without causing fear of death. Or it is very possible that all of these factors may combine to give rise to asphyxial hyperglycemia. But however this may be, the asphyxial blood seems to possess in itself hyperglycemic ability. This ability is neither due to the excess of sugar contained in it, nor to its acidity.



In order to explain this experimental fact, the hypothesis that adrenalin is responsible is most acceptable because we do not know at present any other substance than adrenalin in the blood which can give rise to the enhanced sugar content, though, of course, we can not venture to claim that hyperadrenalinemia was proved by our experiment to exist in asphyxia, for some hitherto unknown agent may be discovered in the future to have been responsible for the effect obtained.

#### SUMMARY

1. The transfusion of the normal blood from rabbits to rabbits has no remarkable effect upon the blood sugar percentage.
2. The transfusion of the asphyxial blood causes a rise in the sugar content of the blood of recipients.
3. The excess of sugar content in the asphyxial blood is not responsible for this increase of sugar percentage in the blood of the recipients.
4. The neutralization of the blood with  $\text{Na}_2\text{CO}_3$  solution does not abolish this property of asphyxial blood.

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## UREA EXCRETION AFTER SUPRARENALECTOMY

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The rate of urea excretion has been shown to be primarily a function of the concentration of urea in the blood (1). But if we divide the urea excretion per hour by the amount of urea in 100 cc. of blood (Addis' ratio, (2) ) and thereby get an expression of the rate of excretion per unit concentration, we find that, in rabbits, this rate of excretion per unit concentration presents a rather wide range of variability—in other words, that for any given concentration of urea in the blood the rate of excretion may be either rapid or slow. This variability must be accounted for by assuming the operation of factors other than the concentration of urea in the blood (3) and the quantity of functioning renal tissue (4). It has further been shown that the subcutaneous injection of epinephrin has the same effect as those factors which *increase* the rate of urea excretion for a given blood concentration (5), and that the subcutaneous injection of pituitrin affects this ratio by *depressing* it (6), that is, the rate of urea excretion at any given blood urea concentration is accelerated after the injection of epinephrin and depressed after the injection of pituitrin. By mixing epinephrin and pituitrin in doses of varying proportions it is possible to get a modified effect of either one, or the doses may be so balanced that the ratio is not affected in either direction (7).

These facts have suggested the hypothesis that an epinephrin-pituitrin balance may exist in the blood which can alter the rate of renal activity in the handling of urea. One way in which the existence of such an epinephrin-pituitrin balance can be investigated is by a double suprarenalectomy which should leave the pituitary effect unopposed. Similarly, removal of the hypophysis cerebri should give an unopposed epinephrin effect. The results of a few not altogether satisfactory experiments on the effect of suprarenalectomy were referred to in a previous communication (7). At that time it was not possible to

obtain more data, but we have recently been able to return to the problem and in this paper present the results of more numerous and better planned experiments.

#### METHODS

Male rabbits were used and the procedure was the same as described previously (1) with the exception that no stomach tube was introduced. Briefly, it consisted of catheterizing the rabbits, which had been kept in the laboratory without food or water since the previous afternoon, at a definite time (9 a.m.) and then collecting the urine by catheterization at the end of the first, second, third and fifth hours. At the middle of each interval 1 cc. samples of blood were obtained from the ear veins. The urea determinations were made with Marshall's urease method, using for the urine the titration method with modifications as detailed by Addis and Watanabe (8) and for the blood the aeration method with the refinements introduced by Barnett (9).

Using this technique the excretion of urea per hour for the various periods, and the corresponding blood urea concentrations were determined, first, for a group of *normal* animals. By dividing the number of milligrams of urea excreted per hour by the number of milligrams of urea in 100 cc. of blood during the same hour, we get the "ratio" (2) or the rate of excretion per unit blood concentration. These values have also been tabulated in our data.

Bilateral lumbar incisions were then made on some of the animals, exposing and manipulating the suprarenals, but not actually removing them, to ascertain any effect of the operation itself, or of the anesthetic, on our ratio curve.

Subsequently the suprarenal glands of these rabbits were removed through lumbar incisions under ether anesthesia. At first we attempted to remove both glands on the same day and immediately follow the operation by a determination of the rate of urea excretion in the same manner as we had done on the normal animals. These animals often died in an extremely exhausted condition within a few hours and often during the course of the experiment. Often kidney function was almost completely depressed in these dying animals. Evidently such a condition of shock would be accompanied by renal disturbances not representative of the true effect of suprarenal removal. After considerable experimenting we concluded that the removal of one gland at a time with an interval of several days between operations and a rest of a day after the final operation before we commenced the



procedure of collecting the urine and blood, gave the most dependable results, coinciding with the findings of others (10), (11). A large percentage of our animals survived, especially after we became more expert in the technique of the operation. The rabbits were under anesthesia from 20 to 25 minutes. On the day following the final operation they appeared to be about as lively and vigorous as the normal animals. The rate of excretion was determined by our procedure then, and on several subsequent occasions.

#### THE SUPRARENAL GLANDS IN THE RABBIT

Since tissues similar to those found within the suprarenal capsules are found elsewhere in the bodies of some animals, the question arises as to just what we remove when we excise these capsules from the rabbit. The comparative anatomy has been discussed quite fully, and also some of the general results of suprarenalectomy, by Biedl (12) and earlier by Tizzoni (13). Both the cortical and the medullary tissues of the suprarenal may occur separately outside of the capsules in the rabbit as in nearly all of the animals having definite, separate, isolated glands. Medullary or chromaffine cells are found in the ganglia of the abdominal sympathetic and in the carotid ganglion. Stilling (14) observed that extirpation of one gland in the rabbit was followed by great hypertrophy of the other gland and of any remnants of the glands which happened to be left at the time of operation. Also that accessory suprarenals are frequently found after ablation of one gland, probably due to a marked proliferation of the isolated patches of this tissue which occur along the vena cava and in other parts. Fulk and Mac'cod (15) have shown that retroperitoneal chromaphil tissue is the same as that of the suprarenal capsules, and that extracts of it have the same reactions as the medullary tissue.

#### DATA

In figure 1 we have plotted curves (from table 1) showing the average excretion of urea in milligrams, the average concentration of urea per 100 cc. of blood, and the average ratio for each of the periods of our experiment, for a series of twenty normal animals before operation. As has been previously mentioned, the ratio =

$$\frac{\text{Urea excreted per hour in mgm.}}{\text{Mgm. of urea in 100 cc. of blood.}}$$

TABLE I  
*Normal animals*

| RABBIT         | UREA EXCRETED PER HOUR IN GRAMS FOR THE FOUR PERIODS |         |         |                   | UREA IN 100 CC. OF BLOOD IN GRAMS |         |         |                   | RATIO    |         |         |                   |
|----------------|--|---------|---------|-------------------|-----------------------------------|---------|---------|-------------------|----------|---------|---------|-------------------|
|                | 1st hour   | 2d hour | 3d hour | 4th and 5th hours | 1st hour                          | 2d hour | 3d hour | 4th and 5th hours | 1st hour | 2d hour | 3d hour | 4th and 5th hours |
| 93             | 0.020  | 0.039   | 0.058   | 0.075             | 0.045                             | 0.043   | 0.051   | 0.058             | 0.45     | 0.91    | 1.15    | 1.29              |
| 121            | 0.032  | 0.038   | 0.017   | 0.042             | 0.033                             | 0.032   | 0.030   | 0.035             | 0.91     | 1.19    | 0.39    | 1.21              |
| 121            | 0.026  | 0.032   | 0.027   | 0.029             | 0.033                             | 0.030   | 0.030   | 0.027             | 0.80     | 1.07    | 0.88    | 1.09              |
| 122            | 0.013  | 0.013   | 0.021   | 0.028             | 0.020                             | 0.023   | 0.021   | 0.022             | 0.67     | 0.58    | 1.02    | 1.27              |
| 127            | 0.084  | 0.112   | 0.116   | 0.123             | 0.105                             | 0.143   | 0.145   | 0.148             | 0.79     | 0.80    | 0.80    | 0.83              |
| 134            | 0.008  | 0.059   | 0.066   | 0.069             | 0.030                             | 0.033   | 0.030   | 0.036             | 0.27     | 1.78    | 2.18    | 1.93              |
| 137            | 0.023  | 0.013   | 0.010   | 0.037             | 0.032                             | 0.032   | 0.035   | 0.036             | 0.69     | 0.41    | 0.33    | 1.00              |
| 138            | 0.026  | 0.024   | 0.038   | 0.035             | 0.042                             | 0.042   | 0.042   | 0.045             | 0.65     | 0.57    | 0.91    | 0.80              |
| 139            | 0.024  | 0.023   | 0.028   | 0.030             | 0.034                             | 0.035   | 0.040   | 0.039             | 0.69     | 0.67    | 0.70    | 0.79              |
| 142            | 0.005  | 0.007   | 0.022   | 0.024             | 0.031                             | 0.027   | 0.057   | 0.041             | 0.17     | 0.24    | 0.40    | 0.60              |
| 51             | 0.009  | 0.029   | 0.035   | 0.051             | 0.057                             | 0.048   | 0.042   | 0.051             | 0.15     | 0.60    | 0.84    | 1.00              |
| 51             | 0.046  | 0.076   | 0.085   | 0.103             | 0.039                             | 0.048   | 0.048   | 0.040             | 1.17     | 1.59    | 1.74    | 2.60              |
| 52             | 0.023  | 0.023   | 0.013   | 0.046             | 0.049                             | 0.035   | 0.052   | 0.046             | 0.46     | 0.65    | 0.24    | 1.00              |
| 52             | 0.018  | 0.026   | 0.054   | 0.086             | 0.033                             | 0.037   | 0.036   | 0.039             | 0.53     | 0.72    | 1.51    | 2.20              |
| 53             | 0.031  | 0.032   | 0.044   | 0.060             | 0.027                             | 0.037   | 0.043   | 0.045             | 1.14     | 0.88    | 1.03    | 1.34              |
| 56             | 0.029  | 0.027   | 0.021   | 0.039             | 0.033                             | 0.038   | 0.040   | 0.030             | 0.89     | 0.71    | 0.53    | 1.30              |
| 58             | 0.024  | 0.028   | 0.031   | 0.039             | 0.038                             | 0.033   | 0.028   | 0.035             | 0.63     | 0.86    | 1.12    | 1.12              |
| 60             | 0.004  | 0.004   | 0.015   | 0.017             | 0.037                             | 0.034   | 0.036   | 0.036             | 0.12     | 0.13    | 0.42    | 0.47              |
| 61             | 0.007  | 0.004   | 0.016   | 0.015             | 0.022                             | 0.024   | 0.027   | 0.038             | 0.34     | 0.19    | 0.58    | 0.41              |
| 62             | 0.013  | 0.009   | 0.013   | 0.015             | 0.024                             | 0.024   | 0.021   | 0.030             | 0.56     | 0.38    | 0.64    | 0.50              |
|                | 0.465  | 0.618   | 0.730   | 0.963             | 0.764                             | 0.798   | 0.855   | 0.877             | 12.08    | 14.93   | 17.41   | 22.75             |
| Average        | 0.023  | 0.031   | 0.037   | 0.048             | 0.038                             | 0.040   | 0.043   | 0.044             | 0.61     | 0.75    | 0.87    | 1.14              |
| Probable error |  |         |         |                   |                                   |         |         |                   | ±0.19    | ±0.27   | ±0.33   | ±0.38             |

It will be observed that the ratio becomes higher—that the rate of excretion of urea per unit concentration of urea in the blood increases—as the experiment progresses, being highest in the last hour. It will be convenient for us, in this discussion, to refer to this tendency for the curve to become higher in the successive periods, as the “epinephrin effect” since it was found to be most marked after the injection of epinephrin (5).

Opposite the curves for normals are drawn similar curves representing the averages of seventeen experiments after both glands had been removed. The ratio curve here does not rise, that is, we do not find

the "epinephrin effect" that occurs in normal animals. The data for this curve are tabulated in table 2.

Figures 3 and 4 are taken from papers already referred to (3), (5) and are introduced for comparison. They show the effects, respectively, of

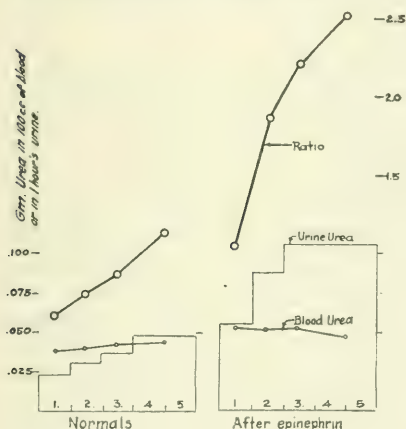


Fig. 1

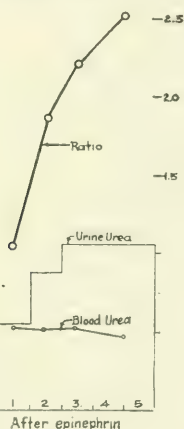


Fig. 2

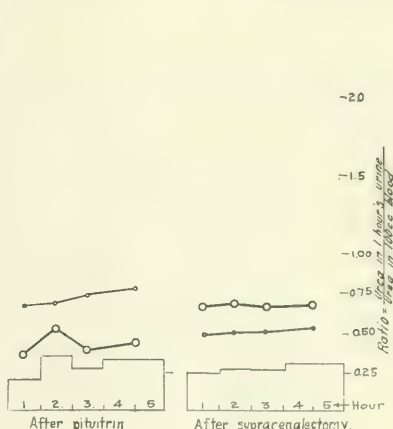


Fig. 3

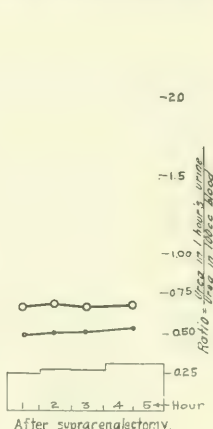


Fig. 4

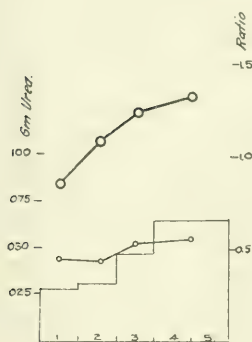


Fig. 5

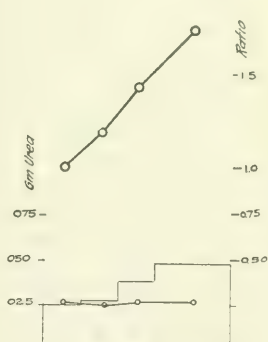


Fig. 6

injecting, subcutaneously, 0.25 cc. Parke, Davis & Company adrenalin at the beginning of each hour, and 0.25 cc. of the same company's pituitrin. The ratio curve rises most markedly after epinephrin and is depressed after pituitrin.

Figure 5 gives the curves for the average of six experiments (table 3) performed on the same day as the *control operation* when the glands were exposed and manipulated but not removed, and figure 6 shows the results of determinations made on some of these same animals on the sixth and seventh days after the control operation. Evidently

TABLE 2

*A compilation of all observations made after both suprarenal glands had been removed. They were made on the day following the removal of the final gland or later.*

| RABBIT                         | UREA, IN GRAMS,<br>EXCRETED PER HOUR |            |            |                            | GRAMS OF UREA IN 100<br>CC. OF BLOOD |            |            |                            | RATIO       |            |            |                            |
|--------------------------------|--------------------------------------|------------|------------|----------------------------|--------------------------------------|------------|------------|----------------------------|-------------|------------|------------|----------------------------|
|                                | 1st<br>hour                          | 2d<br>hour | 3d<br>hour | 4th<br>and<br>5th<br>hours | 1st<br>hour                          | 2d<br>hour | 3d<br>hour | 4th<br>and<br>5th<br>hours | 1st<br>hour | 2d<br>hour | 3d<br>hour | 4th<br>and<br>5th<br>hours |
| 134                            | 0.034                                | 0.029      | 0.035      | 0.039                      | 0.043                                | 0.042      | 0.045      | 0.047                      | 0.78        | 0.68       | 0.77       | 0.82                       |
| 134                            | 0.026                                | 0.024      | 0.032      | Lost                       | 0.024                                | 0.024      | 0.026      | 0.027                      | 1.10        | 1.00       | 1.25       |                            |
| 138                            | 0.033                                | 0.040      | 0.035      | 0.047                      | 0.036                                | 0.048      | 0.045      | 0.039                      | 0.92        | 0.83       | 0.80       | 1.20                       |
| 51                             | 0.037                                | 0.040      | 0.036      | 0.047                      | 0.036                                | 0.036      | 0.036      | 0.040                      | 1.03        | 1.10       | 1.00       | 1.15                       |
| 52                             | 0.010                                | 0.019      | 0.009      | 0.008                      | 0.043                                | 0.042      | 0.045      | 0.042                      | 0.24        | 0.45       | 0.20       | 0.18                       |
| 58                             | 0.010                                | 0.021      | 0.013      | 0.019                      | 0.030                                | 0.037      | 0.040      | 0.038                      | 0.25        | 0.57       | 0.32       | 0.50                       |
| 60                             | 0.012                                | 0.007      | 0.001      | 0.011                      | 0.057                                | 0.056      | 0.051      | 0.048                      | 0.21        | 0.12       | 0.02       | 0.23                       |
| 61                             | 0.056                                | 0.052      | 0.056      | 0.049                      | 0.045                                | 0.045      | 0.036      | 0.042                      | 1.27        | 1.15       | 1.53       | 1.16                       |
| 62                             | Lost                                 | 0.031      | 0.032      | 0.035                      | 0.045                                | 0.042      | 0.039      | 0.042                      |             | 0.75       | 0.81       | 0.83                       |
| 51                             | 0.049                                | 0.041      | 0.060      | 0.063                      | 0.047                                | 0.043      | 0.048      | 0.051                      | 1.04        | 0.96       | 1.25       | 1.23                       |
| 52                             | 0.063                                | 0.055      | 0.075      | 0.068                      | 0.076                                | 0.081      | 0.085      | 0.092                      | 0.82        | 0.68       | 0.88       | 0.74                       |
| 58                             | 0.010                                | 0.008      | 0.001      | 0.001                      | 0.019                                | 0.031      | 0.025      | 0.029                      | 0.55        | 0.26       | 0.04       | 0.04                       |
| 60                             | 0.024                                | 0.017      | 0.019      | 0.014                      | 0.037                                | 0.042      | 0.038      | 0.039                      | 0.65        | 0.41       | 0.51       | 0.37                       |
| 61                             | 0.010                                | 0.011      | 0.009      | 0.008                      | 0.032                                | 0.030      | 0.029      | 0.027                      | 0.33        | 0.35       | 0.31       | 0.29                       |
| 62                             | 0.016                                | 0.020      | 0.007      | 0.001                      | 0.072                                | 0.078      | 0.082      | 0.096                      | 0.23        | 0.23       | 0.08       | 0.02                       |
| 134                            | 0.029                                | 0.047      | 0.040      | 0.065                      | 0.028                                | 0.024      | 0.027      | 0.034                      | 1.05        | 1.96       | 1.50       | 1.90                       |
| 138                            | 0.029                                | 0.035      | 0.035      | 0.053                      | 0.042                                | 0.042      | 0.045      | 0.042                      | 0.68        | 0.84       | 0.77       | 1.27                       |
|                                | 0.448                                | 0.497      | 0.495      | 0.528                      | 0.712                                | 0.743      | 0.742      | 0.775                      | 11.15       | 12.34      | 12.04      | 11.93                      |
| Average                        | 0.028                                | 0.029      | 0.029      | 0.033                      | 0.042                                | 0.044      | 0.044      | 0.045                      | 0.70        | 0.73       | 0.71       | 0.74                       |
| Normal                         | 0.023                                | 0.031      | 0.037      | 0.048                      | 0.038                                | 0.040      | 0.043      | 0.044                      | 0.61        | 0.75       | 0.87       | 1.14                       |
| Difference between ratios..... |                                      |            |            |                            |                                      |            |            |                            | +0.09       | -0.02      | -0.16      | -0.40                      |

any differences in the ratio curves from the normal, due to the anesthetic or to the operation per se, lie entirely within the range of variability of the series and are not measureable. We are, then, justified in interpreting the differences in the curves before and after supra-renalectomy as due to the loss of the glands.



TABLE 3

*These data were obtained on the same day as the control operations, when lumbar incisions were made and the glands exposed and manipulated but not removed. The purpose is to ascertain the effect of the anesthetic and the operation per se, as differentiated from the removal of the suprarenals.*

| RABBIT       | UREA EXCRETED PER HOUR IN GRAMS |         |         |                   | UREA IN 100 CC. OF BLOOD, IN GRAMS |         |         |                   | RATIO    |         |         |                   |
|--------------|---------------------------------|---------|---------|-------------------|------------------------------------|---------|---------|-------------------|----------|---------|---------|-------------------|
|              | 1st hour                        | 2d hour | 3d hour | 4th and 5th hours | 1st hour                           | 2d hour | 3d hour | 4th and 5th hours | 1st hour | 2d hour | 3d hour | 4th and 5th hours |
| 93           | 0.007                           | 0.007   | 0.024   | 0.054             | 0.045                              | 0.045   | 0.075   | 0.060             | 0.15     | 0.15    | 0.32    | 0.89              |
| 116          | 0.049                           | 0.043   | 0.068   | 0.067             | 0.043                              | 0.041   | 0.042   | 0.039             | 1.13     | 1.06    | 1.62    | 1.72              |
| 117          | 0.046                           | 0.051   | 0.062   | 0.049             | 0.030                              | 0.028   | 0.030   | 0.043             | 1.51     | 1.81    | 2.08    | 1.14              |
| 121          | 0.010                           | 0.021   | 0.035   | 0.050             | 0.036                              | 0.038   | 0.045   | 0.044             | 0.29     | 0.56    | 0.77    | 1.13              |
| 122          | 0.037                           | 0.038   | 0.043   | 0.099             | 0.100                              | 0.100   | 0.108   | 0.110             | 0.37     | 0.38    | 0.40    | 0.90              |
| 134          | 0.020                           | 0.023   | 0.048   | 0.072             | 0.012                              | 0.009   | 0.021   | 0.034             | 1.65     | 2.53    | 2.24    | 2.10              |
| Average..... | 0.028                           | 0.031   | 0.047   | 0.065             | 0.044                              | 0.043   | 0.053   | 0.055             | 0.85     | 1.08    | 1.24    | 1.32              |

TABLE 4

*Observations made on the sixth and seventh days after the control operation. In both this table and table 3 it will be noted that the values of the ratio for the successive periods continually increase after the manner of normal animals*

| RABBIT       | GRAMS OF UREA EXCRETED PER HOUR |         |         |                   | GRAMS OF UREA IN 100 CC. OF BLOOD |         |         |                   | RATIO    |         |         |                   |
|--------------|---------------------------------|---------|---------|-------------------|-----------------------------------|---------|---------|-------------------|----------|---------|---------|-------------------|
|              | 1st hour                        | 2d hour | 3d hour | 4th and 5th hours | 1st hour                          | 2d hour | 3d hour | 4th and 5th hours | 1st hour | 2d hour | 3d hour | 4th and 5th hours |
| 93           | 0.012                           | 0.014   | 0.044   | 0.065             | 0.033                             | 0.033   | 0.036   | 0.033             | 0.36     | 0.44    | 1.23    | 1.97              |
| 116          | 0.016                           | 0.021   | 0.019   | 0.023             | 0.020                             | 0.015   | 0.017   | 0.019             | 0.81     | 1.42    | 1.13    | 1.20              |
| 117          | 0.044                           | 0.044   | 0.052   | 0.055             | 0.024                             | 0.026   | 0.027   | 0.026             | 1.84     | 1.70    | 1.93    | 2.03              |
| Average..... | 0.024                           | 0.027   | 0.038   | 0.047             | 0.026                             | 0.025   | 0.027   | 0.026             | 1.00     | 1.19    | 1.43    | 1.73              |

Nine observations on rabbits after the removal of the right gland and before the removal of the left one, gave averages shown in figure 7 and table 5. The ratio curve presents a distinct upward tendency but is slightly less pronounced than in the normal animals. These findings are introduced merely for the sake of completeness; they seem to indicate that the remaining gland is ample to supply the needs of the body.

Figures 8 and 9 are constructed, respectively, from observations made from 24 to 48 hours after the removal of the final gland, and

TABLE 5

*These observations were made on the same day or the next day after the removal of the right suprarenal gland and before the excision of the left gland, in order to ascertain the renal behavior of an animal with only one suprarenal. If there is any effect it is too small to be definite*

| RABBIT      | UREA EXCRETED PER HOUR IN GRAMS |         |         |                   | UREA IN 100 CC. OF BLOOD, IN GRAMS |         |         |                   | RATIO    |         |         |                   |
|-------------|---------------------------------|---------|---------|-------------------|------------------------------------|---------|---------|-------------------|----------|---------|---------|-------------------|
|             | 1st hour                        | 2d hour | 3d hour | 4th and 5th hours | 1st hour                           | 2d hour | 3d hour | 4th and 5th hours | 1st hour | 2d hour | 3d hour | 4th and 5th hours |
| 127         | 0.002                           | 0.006   | 0.014   | 0.014             | 0.046                              | 0.046   | 0.054   | 0.032             | 0.05     | 0.14    | 0.25    | 0.32              |
| 137         | 0.002                           | 0.004   | Lost    | 0.014             | 0.018                              | 0.008   | 0.023   | 0.039             | 0.08     | 0.56    |         | 0.36              |
| 138         | 0.009                           | 0.009   | 0.024   | 0.017             | 0.060                              | 0.054   | 0.064   | 0.075             | 0.15     | 0.17    | 0.37    | 0.24              |
| 139         | 0.003                           | 0.020   | 0.014   | 0.024             | 0.039                              | 0.036   | 0.038   | 0.049             | 0.08     | 0.54    | 0.37    | 0.50              |
| 141         | 0.002                           | 0.003   | 0.007   | 0.018             | 0.030                              | 0.033   | 0.033   | 0.037             | 0.07     | 0.09    | 0.23    | 0.46              |
| 143         | 0.027                           | 0.006   | 0.030   | 0.035             | 0.039                              | 0.042   | 0.047   | 0.066             | 0.70     | 0.14    | 0.64    | 0.53              |
| 51          | 0.005                           | 0.043   | 0.030   | 0.051             | 0.015                              | 0.025   | 0.042   | 0.048             | 0.30     | 1.78    | 0.95    | 1.07              |
| 52          | 0.019                           | 0.019   | 0.030   | 0.050             | 0.033                              | 0.046   | 0.049   | 0.056             | 0.58     | 0.42    | 0.62    | 0.89              |
| 62          | 0.035                           | 0.044   | 0.041   | 0.051             | 0.039                              | 0.040   | 0.042   | 0.036             | 0.88     | 1.07    | 0.99    | 1.40              |
| Average.... | 0.012                           | 0.017   | 0.025   | 0.030             | 0.035                              | 0.037   | 0.041   | 0.049             | 0.32     | 0.54    | 0.55    | 0.64              |

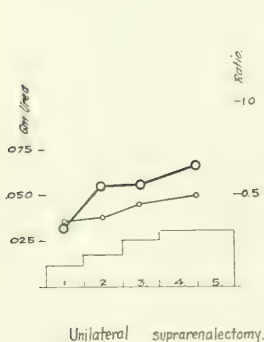


Fig. 7

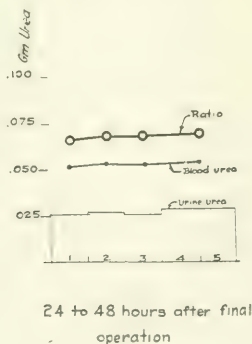


Fig. 8

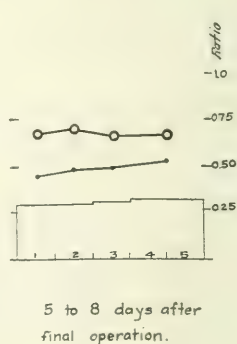


Fig. 9

from 5 to 8 days after the last operation. There is no difference between these curves, indicating that if there is a readjustment of the "balance" after the loss of the suprarenals, it does not occur very soon.

Figure 10 is a graphical summary comparing the present results with some previously obtained. The base line represents the average ratio during the first hour for a group of normal animals. It is the

average of observations on fifty-seven rabbits and its value is 0.69. The rest of the curve is obtained by computing the percentage of this quantity by which the ratio is increased or decreased during the following hours of the experiment and under the varying conditions of it. In the curve for "normals" we have the percentage by which the ratio was increased after the first hour. The curve for "epinephrin" was

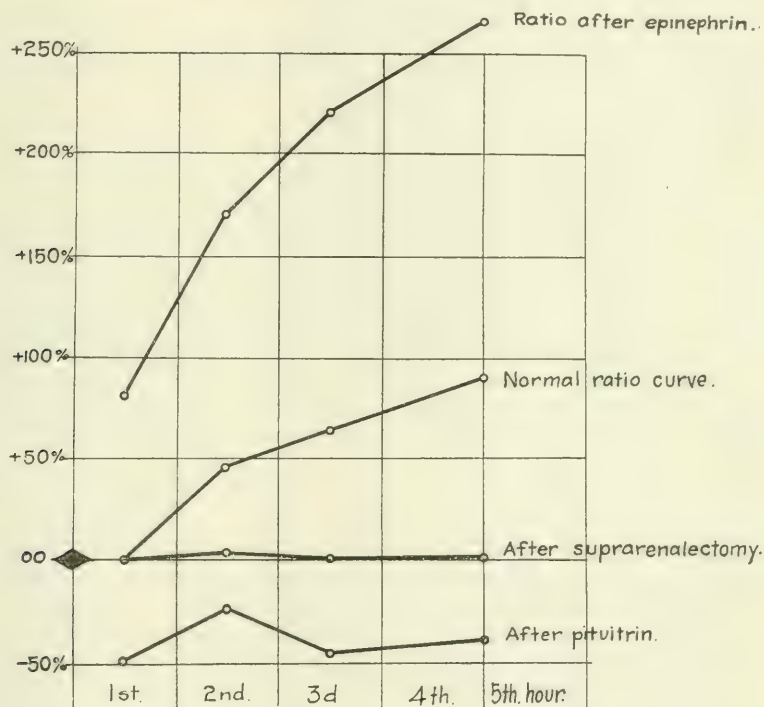


Fig. 10. Chart showing the percentage increase or decrease in the average "ratio" for the various periods of the experiments and under the various conditions listed, compared with the average "ratio" for the first period obtained from 57 normal animals. See table 8.

obtained from data recorded by Addis, Barnett and Shevky (5) and shows the marked increase obtained after the subcutaneous injection of 0.25 cc. Parke, Davis & Company adrenalin at the beginning of each hour, in percentage of the initial ratio for the first hour for animals under normal conditions. It will be noted that there was an increase in the average ratio of about 80 per cent as the result of the first injec-

TABLE 6

*In this table we have collected those observations which were made from twenty-four to forty-eight hours after the excision of the final suprarenal capsule. It maybe compared with the next table in which we have collected observations made from five to eight days after the removal of the last gland*

| RABBIT       | UREA EXCRETED PER HOUR IN GRAMS |         |         |                   | UREA IN 100 CC. OF BLOOD |         |         |                   | RATIO    |         |         |                   |
|--------------|---------------------------------|---------|---------|-------------------|--------------------------|---------|---------|-------------------|----------|---------|---------|-------------------|
|              | 1st hour                        | 2d hour | 3d hour | 4th and 5th hours | 1st hour                 | 2d hour | 3d hour | 4th and 5th hours | 1st hour | 2d hour | 3d hour | 4th and 5th hours |
| 134          | 0.034                           | 0.029   | 0.035   | 0.039             | 0.043                    | 0.042   | 0.045   | 0.047             | 0.78     | 0.68    | 0.77    | 0.82              |
| 134          | 0.027                           | 0.024   | 0.033   | Lost              | 0.024                    | 0.024   | 0.026   | 0.027             | 1.10     | 1.00    | 1.25    |                   |
| 138          | 0.033                           | 0.040   | 0.035   | 0.047             | 0.036                    | 0.048   | 0.045   | 0.039             | 0.92     | 0.83    | 0.80    | 1.20              |
| 51           | 0.037                           | 0.040   | 0.036   | 0.047             | 0.036                    | 0.036   | 0.036   | 0.040             | 1.03     | 1.10    | 1.00    | 1.15              |
| 52           | 0.010                           | 0.019   | 0.010   | 0.008             | 0.043                    | 0.042   | 0.045   | 0.042             | 0.24     | 0.45    | 0.20    | 0.18              |
| 58           | 0.010                           | 0.021   | 0.013   | 0.019             | 0.039                    | 0.037   | 0.040   | 0.038             | 0.25     | 0.57    | 0.32    | 0.50              |
| 60           | 0.012                           | 0.001   | 0.001   | 0.011             | 0.057                    | 0.056   | 0.051   | 0.048             | 0.21     | 0.12    | 0.02    | 0.23              |
| 61           | 0.056                           | 0.052   | 0.056   | 0.049             | 0.044                    | 0.045   | 0.036   | 0.042             | 1.27     | 1.15    | 1.53    | 1.16              |
| 62           | Lost                            | 0.032   | 0.032   | 0.035             | 0.045                    | 0.042   | 0.039   | 0.042             |          | 0.75    | 0.81    | 0.83              |
|              | 0.219                           | 0.258   | 0.251   | 0.255             | 0.367                    | 0.372   | 0.363   | 0.365             | 5.80     | 6.65    | 6.70    | 6.07              |
| Average..... | 0.027                           | 0.029   | 0.028   | 0.020             | 0.041                    | 0.041   | 0.040   | 0.041             | 0.73     | 0.75    | 0.74    | 0.76              |

TABLE 7

*Data obtained from five to eight days after the excision of the last suprarenal capsule*

| RABBIT       | UREA EXCRETED PER HOUR IN GRAMS |         |         |                   | UREA IN 100 CC. OF BLOOD, IN GRAMS |         |         |                   | RATIO    |         |         |                   |
|--------------|---------------------------------|---------|---------|-------------------|------------------------------------|---------|---------|-------------------|----------|---------|---------|-------------------|
|              | 1st hour                        | 2d hour | 3d hour | 4th and 5th hours | 1st hour                           | 2d hour | 3d hour | 4th and 5th hours | 1st hour | 2d hour | 3d hour | 4th and 5th hours |
| 51           | 0.049                           | 0.041   | 0.060   | 0.063             | 0.047                              | 0.043   | 0.048   | 0.051             | 1.04     | 0.96    | 1.25    | 1.23              |
| 52           | 0.062                           | 0.055   | 0.075   | 0.068             | 0.076                              | 0.081   | 0.085   | 0.092             | 0.82     | 0.68    | 0.88    | 0.74              |
| 58           | 0.011                           | 0.008   | 0.001   | 0.001             | 0.019                              | 0.031   | 0.025   | 0.029             | 0.55     | 0.26    | 0.04    | 0.04              |
| 60           | 0.024                           | 0.017   | 0.020   | 0.014             | 0.037                              | 0.042   | 0.038   | 0.039             | 0.65     | 0.41    | 0.51    | 0.37              |
| 61           | 0.010                           | 0.010   | 0.009   | 0.008             | 0.032                              | 0.030   | 0.029   | 0.027             | 0.33     | 0.35    | 0.31    | 0.29              |
| 62           | 0.016                           | 0.020   | 0.007   | 0.002             | 0.072                              | 0.078   | 0.082   | 0.096             | 0.23     | 0.23    | 0.08    | 0.02              |
| 34           | 0.029                           | 0.047   | 0.041   | 0.064             | 0.028                              | 0.024   | 0.027   | 0.034             | 1.05     | 1.96    | 1.50    | 1.90              |
|              | 0.201                           | 0.198   | 0.213   | 0.220             | 0.311                              | 0.329   | 0.334   | 0.368             | 4.67     | 4.83    | 4.57    | 4.55              |
| Average..... | 0.029                           | 0.029   | 0.030   | 0.032             | 0.044                              | 0.047   | 0.048   | 0.052             | 0.67     | 0.69    | 0.65    | 0.66              |



tion of adrenalin and that this became greater as the hours passed. The curve for pituitrin shows that the subcutaneous injection of pituitrin at the beginning of each hour depressed the rate of excretion per unit blood concentration. Similarly our results after suprarenalectomy show that the progressive increase detected in normal animals with intact glands, or after the injection of epinephrin, does not occur.

It might be noted that the urea concentration in the blood remains fairly constant under the various conditions of the experiments. It was only in the moribund animals after simultaneous double supra-

TABLE 8

|   | HOURS  |        |        |        |
|---|--------|--------|--------|--------|
|   | 1      | 2      | 3      | 4 to 5 |
| Normals: average of 57 animals.....     | 0.69   | 1.01   | 1.13   | 1.31   |
| Percentage increase over first hour...  | 00     | 46.0%  | 64.0%  | 90.0%  |
| After epinephrin (28 animals).....      | 1.04   | 1.86   | 2.21   | 2.52   |
| Percentage increase over normals....    | 80.0%  | 170.0% | 220.0% | 265.0% |
| After pituitrin (9 animals).....        | 0.25   | 0.52   | 0.38   | 0.43   |
| Percentage decrease.....                | -49.0% | -25.0% | -45.0% | -38.0% |
| After suprarenalectomy (17 animals).... | 0.70   | 0.72   | 0.70   | 0.71   |
| Percentage increase.....                | 1.0%   | 4.0%   | 1.0%   | 3.0%   |

The normal ratio for the first hour of the experiment for a group of fifty-seven rabbits is 0.69. In the second hour it is 1.01 or an increase of 46 per cent and so on for the rest of the periods. After a subcutaneous injection of epinephrin we get an average ratio of 1.04 for the first period or an increase of 80 per cent over the first period excretion for normal animals.

renalectomy that we found an increase in the level of blood urea similar to that described by Marshall and Davis in cats (16). When one gland was removed at a time the rabbits remained more normal.

#### DISCUSSION

Normal rabbits present a progressive increase in the rate of urea excretion during the consecutive periods of our experiment, so that at the end the rate was nearly twice as great as it was in the beginning, in spite of the fact that the urea concentration in the blood remained practically constant. This means, of course, that the kidneys did not have more to do, but performed what they did have to do at a more

rapid rate. The conditions of the experiment were the same at the end as at the beginning except that the animals had undergone considerable handling and discomfort.

The progressive increase found in normal animals may be greatly accentuated by the subcutaneous injection of epinephrin, and may be prevented, or even a progressive decrease may be obtained, by the injection of pituitrin. It has been shown that injections of epinephrin and pituitrin in varying proportions may affect the rate of urea excretion in mutually antagonistic directions and that each may neutralize the effect of the other. And on the basis of these data the hypothesis was advanced that a possible balance between the secretions of the suprarenals and of the hypophysis, in the blood, may be a factor in determining the state of renal activity (7).

The fact that the removal of the suprarenal glands affected the form of the ratio curve in a manner remarkably similar to that produced by the subcutaneous injection of pituitrin (figs. 2 and 4) strongly suggests the existence of such a balance—the effect of suprarenalectomy being an unbalanced pituitary effect.

Marshall and Davis (16) have observed in cats a similar decrease in the rate of excretion of urea, creatinin and chlorides, with which we, of course, agree. Motzfeldt (17) found that the extract or secretion of the posterior lobe of the hypophysis had a strong antidiuretic effect on rabbits, which was most pronounced after 2 to 4 hours. Rees (18) noted that pituitary extracts delay diuresis after ingested water, for 7 or 8 hours, but do not alter the 24-hour volume.

The depression recorded after ablation of the glands is less marked than after the injection of an optimum dose of pituitrin. This is as might be expected, for the excision of the suprarenal capsules has only removed *most*, not all, of the medullary tissue, as previously pointed out. Furthermore, there has been no stimulus to call forth a maximum pituitrin effect, and only the normal amount of pituitary secretion is probably present in the blood. This causes, however, a definite "pituitary effect" for it is not balanced by the full normal suprarenal secretion.

We attribute the progressive increase in the rate of excretion, in spite of constant blood urea concentration, which we found in all normal rabbits during the successive periods of the experiments, to a gradual increase in the rate of secretion of epinephrin from the suprarenal glands. After the removal of the glands such an increase of epinephrin in the blood, of course, could not occur.

## CONCLUSIONS

1. The removal of the suprarenal glands in rabbits is followed by a depression of the rate of urea excretion by the kidneys.

2. The form of the curve obtained by plotting the ratio between the urea excreted per hour and the concentration of urea in the blood, for the various intervals of the experiment, is modified after suprarenalectomy in a manner strikingly like that obtained by the subcutaneous injection of optimum doses of pituitrin, and in a manner contrary to that obtained after the injection of epinephrin.

3. It is suggested that these findings support the hypothesis that an epinephrin-pituitrin balance exists in the blood which may regulate the rate of kidney function, the results obtained after suprarenalectomy exhibiting a pituitary effect unopposed by the normal secretion of the suprarenals.

We wish to acknowledge our indebtedness to Dr. Thomas Addis for his valuable suggestions and for his kindly interest, which has always been most stimulating.

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# POSTURE-SENSE CONDUCTION PATHS IN THE SPINAL CORD

## A PRELIMINARY REPORT

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Exact knowledge is lacking as to how impulses mediating posture-sense are conducted in the spinal cord. These, with such other afferent impulses as muscle-sense, deep-sensibility and others of the sensation-complex are supposed to travel upward, without decussation, in the dorsal and lateral columns of the spinal cord. Decussation of these fibers takes place in the medulla superior to the pyramidal decussation. To obtain more definite information as to the manner of transmission of posture-sense impulses in the spinal cord, we have applied to our problem the animal behavior method described by O. Kalischer<sup>1</sup> in his experiments on audition. By means of a strong "hunger-motif" this investigator trained dogs to discriminate between various tones, permitting them to take food only when the correct or "training-stimulus" was presented. Dogs were trained daily over a period of weeks until they perfectly discriminated the "feeding-stimulus" from any other. This "feeding-stimulus" was always the same tone sounded on the organ. After they had learned the "lesson" the animals were operated and an area of the cerebral cortex was destroyed. After complete recovery from the shock of the operation the dogs were again critically tested to determine their ability to perfectly differentiate the "feeding-stimulus" from other stimuli. If discrimination by the animal was still perfect, conclusion was drawn that the center for reception of the impulse was not destroyed. Likewise the converse was held true: that lost or confused discrimination indicated destruction of a specific center.

In our experiments we applied the same principle to tracts in the spinal cord. A dog was trained to take food only when the right hind

<sup>1</sup>Sitzungsb. d. Königl. Preus. Akad. d. Wissensch., 1907, x.



leg was held in a certain position,—that of rigid extension backward; and to refuse food when the same foot was held in any other position. To eliminate possible habit formation by the dog to the stimulus of pressure on the leg, in both phases of the training (extension and flexion) the dog's foot was subjected to an equal pressure by the hand of the operator. Unconscious cues and helps were carefully eliminated. In fact, however, due to the extreme hunger of the animal, it would have been almost impossible to distract his attention from the problem. Grown dogs of all breeds were used but the sharp-nosed type served our purpose best. The dogs were never petted, spoken to nor allowed to commingle. In this way was developed a state of loneliness and eagerness for companionship which made them tractable to training and eager for work.

The exact procedure was as follows: The dogs were placed in clean cages and allowed no food for some days. Then by means of the strong "hunger-motif" developed they were taught to leave their cages, to mount three steps to the experiment table, to take a certain position and to wait there until fed. Then the dog's right hind leg was grasped by the operator and extended rigidly backward. A cube of cooked meat was then placed before the dog which he was allowed to take during this phase of the training. This backward extension furnished the "feeding-stimulus" for this animal. Next, the foot was placed in position of rest by the operator, and another cube of meat was offered the dog. During this phase of training the dog was not allowed to seize the particle of food. Only during the first days of the training was it necessary for the operator to interpose his hand between the dog's muzzle and the cube of meat. The dog soon learned when to take food and when to leave it. Punishment was *never* given for mistakes. In our experience such treatment of the dog rendered him unfit for training. As part of the training the animals were taught to return to their cages after feeding. Each daily lesson lasted about eight or ten minutes during which time about fifty equal-sized cubes of meat were fed to the dog. Great care was taken not to impair the dog's "hunger-motif," either by over-feeding at the daily lesson or by feeding between lessons. The dogs remained healthy during the experiment but became somewhat emaciated.

The following precautions were taken in our training experiments:

1. Dogs were never petted, spoken to nor punished throughout the course of the training.

2. Dogs were never over-fed nor fed at any other time than training time. In this way a strong "hunger-motif" was maintained. This is the key to the experiment and eliminates such distracting factors as inattention, indifference or fatigue.

3. Duration of time of stimuli was the same and the order of presentation varied by daily rearrangement. This precaution was taken to prevent rhythmic habit formation which might occur if the order of presentation of stimuli were left to chance.

The dogs were considered perfectly trained after they had been taught to differentiate without error the "feeding-stimulus" from all others. They were tested with the "feeding-stimulus" and other stimuli fifteen to thirty times at the daily lesson over a period of two or three weeks. After they were found perfect, the spinal cord was hemisected on the right side about the level of the first thoracic vertebra. Laminectomy was done under ether anesthesia and the cord carefully and completely exposed. Then the dura was incised and the wound packed for a few minutes. After a dry field was secured the cord was carefully hemisected.

After recovery from the shock of the operation, usually the second or third day, discrimination tests were again undertaken, similar to those used during the training of the animal. These tests were carried out over a period of from three to six weeks and were very satisfactory because of the prompt and decided responses of the animals to the stimuli employed. Careful notes on the behavior of the animals were made and will form the basis of full protocols in a later paper.

At the end of six weeks the dogs were killed, the cords removed and the gross hemisection noted. The cords were then preserved in Müller's fluid for histological study of the degenerations. Marchi's method will be used.

#### SUMMARY

*Dog 1.* Trained to accept food with right hind leg rigidly extended. *Right* hemisection of cord about level of first thoracic vertebra. Responses to posture-tests in right hind leg prompt and decided. Motor paralysis right hind leg complete. Pain sense lost in right hind leg.

*Dog 2.* Trained like dog 1. *Right* hemisection of cord about the level of the last thoracic vertebra; and two months later another *right* hemisection of cord about the level of the first lumbar vertebra. Responses to posture-tests in right hind leg prompt and decided. Motor paralysis right hind leg complete. Pain sense lost in right hind leg.

*Dog 3.* Trained like dogs 1 and 2. *Right* hemisection of cord at level of first thoracic vertebra and two months later *left* hemisection at level of first lumbar vertebra. Responses to posture-tests in right hind leg prompt and decided. Motor paralysis of both hind legs complete.

It was suggested that perhaps the animal was acting upon cutaneous stimuli alone; to eliminate this factor we blocked the cutaneous nerves of the right hind extremity of one animal by injecting a 2.0 per cent solution of cocaine into the skin close to the trunk. Pain sense was then tested with a red hot wire and the animal made no response. The response was unmistakable when the left side was tested.

#### CONCLUSIONS

1. That decussation of *part* of the fibers mediating posture-sense impulses occurs within the cord.
2. That some of the impulses mediating posture-sense probably travel back and forth across the cord at different levels by short association fibers.

# STUDIES ON THE REGULATION OF THE BLOOD DIASTASE

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Wohlgemuth (1) reported that the diastase content of the blood is very stable and not influenced by feeding or administration of certain drugs (adrenalin, morphine, etc.), which have a marked effect upon its sugar content. The investigations reported in this paper were carried out in order to determine, if possible, how the blood diastase is regulated.

Clerc and Loeper (2) and Gould and Carlson (3) observed that the ligation of the pancreatic ducts is followed by an increased diastatic activity in the blood serum. They assumed this to be absorbed amylopsin. Otten and Galloway (4) and King (5) found, on the other hand, that the blood diastase sinks rapidly after complete pancreatectomy. The pancreas is, therefore, regarded as the chief source of the blood diastase.

Wohlgemuth (6) stated that the diastatic activity of the blood serum in the portal vein is stronger than that in the hepatic vein. Schlesinger (7) also found that the diastatic power of the blood serum in the pancreatic vein was two or three times stronger than that in the mesenteric vein or the peripheral blood vessel in some cases. Wohlgemuth, in discussing this report of Schlesinger, states that some difference in the diastatic power can be found between the blood in the portal and peripheral veins but not between the blood in the portal and pancreatic veins. Thus it may be said that the diastatic substance passes from the pancreas into the liver and is there mixed with the blood and lymph, its output into the blood being regulated by the liver.

*The diastase content of the blood in the portal vein compared with that in the peripheral blood vessel.* We have compared the diastase content of the blood in the portal vein with that in the peripheral blood vessel (table 1). In our experiments guinea pigs were always employed. For the estimation of diastase, we have employed Wohlgemuth's



method as modified by Inoue (8). The results of the digestion were seen after incubating for 30 minutes in a water bath at 38°C.

In table 1 we see that the diastase content of the blood in the portal vein varies considerably and that sometimes it is larger than that in the peripheral blood vessel. These results confirm the reports of Wohlgenuth and Schlesinger. Hence it can be said with certainty that the diastase content of the blood is regulated by the liver.

*The influence of hepatotoxin upon the diastase content of the blood.* Next we have undertaken to injure the liver-cells by parenteral administration of hepatotoxin to disturb the regulation of diastase in the liver.

TABLE 1

*The diastase content of the blood serum from the portal vein and carotid artery*

| GUINEA PIG<br>NUMBER | DIASTASE D $\frac{30'}{38^\circ}$ |             | REMARKS                          |
|----------------------|-----------------------------------|-------------|----------------------------------|
|                      | Carotid                           | Portal vein |                                  |
| 21                   | 185                               | 150         |                                  |
| 55                   | 125                               | 150         | After feeding                    |
| 56                   | 250                               | 185         | After feeding (V. mesenter. 250) |
| 57                   | 215                               | 215         |                                  |
| 58                   | 125                               | 150         |                                  |
| 59                   | 125                               | 125         |                                  |

To a series of test tubes there were added increasing amounts of the blood serum and a constant dose of the amylum solution. In our experiments the amounts of the blood serum were so graduated that the diastatic activity in each test tube, when the amylum in it is completely digested, shows each 75, 85, 95, 105, 125, 150, 165, 185, 215, 250, 300.

Though Karsner and Aub (9) have brought forth contradictory findings against the view of Delezenne (10), who had affirmed the organ specificity of hepatotoxin, I have recently proved (not yet published) that hepatotoxin acts specifically on the liver-cells and destroys their normal function. Hence the hypothesis that, if the liver is truly a regulator of the blood diastase, the latter may be influenced by the administration of the hepatotoxin.

The hepatotoxin used in our experiments was obtained by immunizing rabbits with emulsions of the liver of the guinea pig. The intraperitoneal injections were repeated twice. Various doses of hepatotoxin thus obtained were injected intraperitoneally in guinea pigs and the diastase content of the blood was examined repeatedly. The blood was always obtained from the ear. The results are shown in table 2.

As we see in table 2, the diastase content of the blood sinks rapidly after injection of the hepatotoxin and then rises in a few days to its normal value.

From these results we are able to say that the liver cells which regulate the diastase content in the blood were intoxicated by hepatotoxin.

*The effect of pancreatotoxin upon the diastase content of the blood.* The foregoing experiments were accompanied by the following tests in order to see if other organ toxins can also influence the diastatic activity of the blood. Pancreatotoxin was prepared by immunizing rabbits with emulsions of the pancreas of the guinea pig. The administration of the pancreatotoxin may cause a degeneration of the pan-

TABLE 2

*The diastatic activity of the blood in the peripheral blood vessel before and after the injection of hepatotoxin*

| GUINEA<br>PIG<br>NUMBER | BODY<br>WEIGHT | AMOUNT<br>OF HEPATO-<br>TOXIN IN-<br>JECTED IN-<br>TO PERI-<br>TONEAL<br>CAVITY | DIASTATIC ACTIVITY $D_{38}^{30'}$ |                                    |     |     |     |     |     |     |
|-------------------------|----------------|---|-----------------------------------|------------------------------------|-----|-----|-----|-----|-----|-----|
|                         |                |   | Before<br>injec-<br>tion          | Day after injection of hepatotoxin |     |     |     |     |     |     |
|                         |                |   |                                   | 3-4<br>(hours)                     | 1   | 2   | 3   | 4   | 5   | 6   |
|                         | <i>grams</i>   |   |                                   |                                    |     |     |     |     |     |     |
| 5                       | 630            | 3   | 165                               |                                    |     | 105 | 125 | 125 |     | 150 |
| 29                      | 570            | 3   | 165                               | 125                                | 105 |     |     |     |     | 165 |
| 31                      | 500            | 3   | 150                               | 125                                | 105 |     |     |     |     | 125 |
| 7                       | 600            | 4   | 185                               | 150                                | 125 |     | 150 |     | 165 |     |
| 9                       | 415            | 4   | 165                               | 125                                | 105 |     | 165 |     |     |     |
| 10                      | 530            | 4   | 165                               | 125                                | 150 |     | 150 |     |     |     |
| 47                      | 730            | 8   | 105                               |                                    | 80  | 105 |     | 105 |     | 125 |
| 46                      | 590            | 10  | 150                               |                                    | 95  | 105 |     | 105 |     | 165 |

creatic cells. Hence supposing that the pancreatotoxin would influence the production of diastase in the pancreas, we have examined the diastatic activity of the blood after the injection of this toxin (see table 3).

As we can see in table 3, no appreciable change in the diastase content of the blood in the peripheral blood vessel was observed after injection of the pancreatotoxin, except in two guinea pigs, which received large doses (8 or 10 cc.). Such a large amount of this toxin was near to the lethal dose for guinea pigs. Therefore, it is assumed that the decrease in the diastase content in these two animals was probably caused by an intoxication of the liver cells.

TABLE 3

*The diastatic activity of the peripheral blood before and after injection of pancreatotoxin*

| GUINEA<br>PIG<br>NUMBER | BODY<br>WEIGHT | AMOUNT OF<br>PANCREA-<br>TOTOXIN IN-<br>JECTED IN-<br>TO PERI-<br>TONEAL<br>CAVITY | DIASTATIC ACTIVITY $D_{38}^{30'}$ |                                       |     |     |     |     |     |   |
|-------------------------|----------------|--|-----------------------------------|---------------------------------------|-----|-----|-----|-----|-----|---|
|                         |                |  | Before<br>injec-<br>tion          | Day after injection of pancreatotoxin |     |     |     |     |     |   |
|                         |                |  |                                   | 3-4<br>(hours)                        | 1   | 2   | 3   | 4   | 5   | 6 |
|                         | <i>grams</i>   |  |                                   |                                       |     |     |     |     |     |   |
| 3                       | 560            | 3  | 185                               | 185                                   |     | 185 |     |     | 185 |   |
| 4                       | 370            | 3  | 215                               | 185                                   |     | 215 |     |     | 185 |   |
| 2                       | 390            | 2  | 185                               | 185                                   | 185 |     | 215 |     | 185 |   |
| 18                      | 710            | 8  | 125                               |                                       | 95  | 125 |     | 150 | 150 |   |
| 19                      | 640            | 10   | 150                               |                                       | 105 | 105 |     | 150 | 165 |   |

In our experiments it was not decided whether the production of diastase in the pancreas was influenced by pancreatotoxin or not. But we can say now that the production or mobilization of diastase in the pancreas was not so much affected that the liver could not regulate the diastase content of the blood in the peripheral blood vessel, and also that the regulating action of the liver was not influenced by a dose of 3 or 4 cc. of this toxin.

Several experiments for control were undertaken with neurotoxin and the blood serum of normal rabbits. The results are shown in table 4.

TABLE 4

*The diastatic activity of the peripheral blood before and after injection of the neurotoxin and the blood serum of normal rabbits*

| GUINEA<br>PIG<br>NUMBER | BODY<br>WEIGHT | AMOUNT<br>OF NEURO-<br>TOXIN OR<br>NORMAL<br>SERUM IN-<br>JECTED IN-<br>TO PERI-<br>TONEAL<br>CAVITY | DIASTATIC ACTIVITY $D_{38}^{30'}$ |  |     |     |     |     |     |     |
|-------------------------|----------------|--|-----------------------------------|--|-----|-----|-----|-----|-----|-----|
|                         |                |  | Before<br>injec-<br>tion          | Day after injection of the neurotoxin or the blood serum of normal rabbits |     |     |     |     |     |     |
|                         |                |  |                                   | 3-4<br>(hours)   | 1   | 2   | 3   | 4   | 5   | 6   |
|                         | <i>grams</i>   |  |                                   |  |     |     |     |     |     |     |
| 33                      | 510            | 3*   | 150                               | 150  | 150 |     |     |     |     | 165 |
| 34                      | 630            | 3*   | 150                               | 150  | 150 |     | 165 |     |     | 150 |
| 35                      | 580            | 3*   | 215                               | 185  | 215 | 215 |     |     |     | 215 |
| 36                      | 490            | 8*   | 165                               | 125  | 105 |     | 125 |     | 165 |     |
| 58                      | 580            | 3†   | 150                               | 125  | 150 | 125 | 125 | 125 |     |     |
| 59                      | 520            | 3†   | 125                               | 125  | 125 | 150 | 125 | 125 |     |     |

\* Neurotoxin.

† Normal serum.

As we see in the above table, 3 to 4 cc. of the neurotoxin or the blood serum of normal rabbits caused no change in the diastase content in the blood, while the same dose of hepatotoxin markedly affected it.

Here we have also exceptions which may, however, be explained in the same manner as in the cases of pancreatotoxin. It can be now concluded that the hepatotoxin attacked the liver and affected its regulating power over the blood diastase.

*The effect of the injection of adrenalin upon the blood diastase of guinea pigs, to which hepatotoxin was intraperitoneally injected.* Starkenstein (1), Allen (12) and Watanabe (14) found no marked change in the

TABLE 5

*The diastatic activity of the blood before and after injection of adrenalin or morphine*

| GUINEA PIGS<br>WHICH WERE<br>TREATED WITH<br>HEPATOTOXIN | BODY WEIGHT  | AMOUNT OF INJECTED ADRENALIN<br>OR MORPHINE | DIASTATIC ACTIVITY                                 |  |
|--|--------------|---|--|--|
|  |              |   | Before injection<br>D <sub>38</sub> <sup>30'</sup> | 2 hours after<br>injection<br>D <sub>38</sub> <sup>30'</sup> |
| <i>number</i>  | <i>grams</i> |   |  |  |
| 11   | 430          | Adrenalin, 0.1 mgm.                         | 250  | 250  |
|  |              | Adrenalin, 0.2 mgm.                         | 250  | 250  |
|  |              | Adrenalin, 0.4 mgm.                         | 185  | 165  |
| 12   | 470          | Adrenalin, 0.15 mgm.                        | 185  | 185  |
|  |              | Adrenalin, 0.25 mgm.                        | 215  | 215  |
|  |              | Adrenalin, 0.5 mgm.                         | 165  | 150  |
| 3  | 560          | Morphine, 0.02 gram                         | 185  | 185  |
| 1  | 500          | Morphine, 0.02 gram                         | 250  | 250  |

diastase content of the blood after subcutaneous or intravenous injections of adrenalin or morphine. As we have proved in the foregoing experiments, the hepatotoxin can injure the liver, which regulates the blood diastase. Supposing, therefore, that adrenalin or morphine might affect the diastase content of the blood of the guinea pigs, which were treated with hepatotoxin, these drugs were subcutaneously injected in them (see table 5). But the diastase content of the blood was never affected.

*The effect of Taka-diastase administered intraperitoneally upon the blood diastase.* The injection of Taka-diastase might cause an increase in the diastatic activity of the blood of guinea pigs, especially after they



have been treated with hepatotoxin. In the following experiments a large dose of Taka-diastase was administered intraperitoneally, and the diastatic activity of the blood was examined (see table 6).

I was surprised to see such a marked decrease in the diastatic activity of the blood in spite of the injection of such a considerable quantity of diastatic substance.

In 1917 Richard Weil (14) proved that peptone markedly affects the liver. Taka-diastase contains peptone, besides diastatic ferment.

TABLE 6

*The diastatic activity of the blood of guinea pigs, which were treated with hepatotoxin, before and after injection of Taka-diastase*

| GUINEA<br>PIG<br>NUM-<br>BER | BODY<br>WEIGHT | AMOUNT<br>OF TAKA-<br>DIASTASE<br>INJECTED<br>INTO PERI-<br>TONEAL<br>CAVITY | DIASTATIC ACTIVITY ( $D_{38}^{30'}$ ) AFTER INJECTION OF TAKA-DIASTASE |         |     |     |     |     |     |     |     |     |
|------------------------------|----------------|--|--|---------|-----|-----|-----|-----|-----|-----|-----|-----|
|                              |                |  | Before<br>injec-<br>tion   | Minutes |     |     |     | Day |     |     |     |     |
|                              |                |  |  | 15      | 30  | 60  | 180 | 1   | 2   | 3   | 4   | 5   |
|                              | <i>grams</i>   |  |  |         |     |     |     |     |     |     |     |     |
| 31*                          | 500            | 0.05   | 125  |         |     |     | 95  | 85  | 125 | 125 | 150 |     |
| 41*                          | 860            | 0.05   | 150  |         |     | 105 | 105 |     | 125 | 125 | 125 | 150 |
| 44*                          | 710            | 0.05   | 165  |         |     | 125 | 105 |     | 105 | 125 | 125 | 150 |
| 45*                          | 730            | 0.5  | 125  |         | 125 |     | 85  | 75  | 105 | 105 | 150 |     |
| 47*                          | 710            | 0.05   | 150  | 105     | 95  |     | 95  | 85  | 95  | 105 | 105 | 125 |
| 15†                          | 610            | 0.05   | 150  |         |     |     | 95  | 85  | 125 | 150 | 150 | 165 |
| 19†                          | 640            | 0.05   | 165  | 150     | 125 | 125 |     | 105 | 125 | 125 |     |     |
| 49‡                          | 550            | 0.05   | 165  |         |     | 125 | 105 |     | 165 | 165 |     |     |
| 50‡                          | 550            | 0.05   | 150  |         |     | 125 | 105 |     | 165 | 185 |     |     |

\* Treated with hepatotoxin.

† Treated with pancreatotoxin.

‡ Not treated.

The latter may be eliminated by heating at 100°C. Hence, if Taka-diastase is heated at 100°C. there will remain the cocto-stable peptone. In the following experiments we have injected the heated Taka-diastase in normal guinea pigs in order to see if it affects the diastatic activity of the blood. Pure peptone dissolved in aqua destillata was also injected in guinea pigs intraperitoneally for control. The results are shown in table 7.

We see now from the table 7 that the heated Taka-diastase and peptone produced similarly a marked change in the blood diastase. The

curves of the diastase content in the blood following injections of hepatotoxin, Taka-diastase and peptone are almost the same. As such a decrease in the diastase content of the blood may be caused by 3 or 4 cc. of hepatotoxin, but not by the same dose of the other organ toxin, we can explain this decrease by assuming that the change in the blood diastase after injections of Taka-diastase and peptone was caused by the intoxication of the liver cells.

TABLE 7

*The diastatic activity of the blood before and after injection of heated Taka-diastase or peptone*

| GUIN-<br>EA<br>PIG<br>NUM-<br>BER | BODY<br>WEIGHT | AMOUNT OF TAKA-DIASTASE OR PEP-<br>TONE INJECTED INTO PERITONEAL<br>CAVITY | DIASTATIC ACTIVITY $D_{38}^{30'}$ |  |     |     |     |     |     |     |
|-----------------------------------|----------------|--|-----------------------------------|--|-----|-----|-----|-----|-----|-----|
|                                   |                |  | Before<br>injec-<br>tion          | Day after injection of heated Taka-<br>diastase or peptone |     |     |     |     |     |     |
|                                   |                |  |                                   | 3-4<br>(hours)   | 1   | 2   | 3   | 4   | 5   | 6   |
|                                   | <i>grams</i>   |  |                                   |  |     |     |     |     |     |     |
| 29                                | 570            | Heated Taka-diastase, 0.05   | 125                               | 95   | 75  | 95  | 125 | 150 |     |     |
| 30                                | 520            | Heated Taka-diastase, 0.05   | 125                               | 105  | 95  | 105 | 125 |     |     |     |
| 55                                | 490            | Peptone, 0.05  | 150                               | 105  | 95  | 95  | 105 | 125 | 125 | 125 |
| 56                                | 620            | Peptone, 0.05  | 125                               | 105  | 95  | 105 | 105 | 125 | 125 |     |
| 57                                | 610            | Peptone, 0.05  | 150                               | 105  | 105 | 105 | 125 | 125 | 150 |     |

## SUMMARY

1. The diastase content of the blood in the portal vein varies, while that in peripheral blood vessel remains constant.

2. Hepatotoxin administered intraperitoneally causes a marked decrease in the diastase content of the peripheral blood, while the same dose of pancreatotoxin or neurotoxin has no effect upon it.

3. Even the intraperitoneal injection of a large dose of Taka-diastase does not cause an increase in the blood diastase; on the contrary, it is followed by a marked decrease in the diastase content of the blood.

4. The diastatic activity of the blood is considerably weakened after the injection of heated Taka-diastase or peptone, which intoxicate the liver cells.

5. It seems sure that the diastase is regulated by the action of the liver cells.

I wish to express my thanks to Prof. Dr. K. Katayama and Prof. Dr. S. Mita for their kind advice.

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## THE CHANGES IN THE CONTENT OF HEMOGLOBIN AND ERYTHROCYTES OF THE BLOOD IN MAN DURING SHORT EXPOSURES TO LOW OXYGEN

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The compensatory changes that occur in the blood of man and animals living under low oxygen tension have held the interest of many investigators. As long ago as 1878 Paul Bert (1) predicted that the blood of those living at high altitudes would be found to have a greater oxygen capacity than the blood of similar individuals living at lower levels, and he further suggested that the cause of the increase would be found to be the decrease in the partial pressure of the oxygen in the air respired. Since that time it has been clearly proved that a decrease in the partial pressure of oxygen of the respired air, regardless of the method of reduction, causes, if the reduction is great enough and the exposure continued through a sufficient interval of time, an increase in the erythrocytes and hemoglobin per unit volume of the blood. This increase has been found to be gradual, requiring from three to five and even more weeks to reach its maximal value (2), (3).

The time required for the increase in erythrocytes and hemoglobin to be first manifest has received some consideration. Campbell and Hoagland (4) carried rabbits to the summit of Pike's Peak, from an altitude of 6000 feet to one of 14,110, and found that in the ascent the number of red corpuscles had made an average increase of 9 per cent. Abderhalden (5), working with rabbits and rats, found an increase within a few hours. Ehrlich and Lazarus (6) state that the increase occurs immediately when considerable altitudes are reached. Douglas, Haldane, Henderson and Schneider (7) found in four men, several hours after their arrival on Pike's Peak, a slight increase in hemoglobin that varied for these individuals from 0.9 to 3.9 per cent. Schneider and Havens (2) were unable to demonstrate a clearly defined increase during the first seven hours spent on Pike's Peak, but within twenty-four hours there was a marked increase in the number of red



corpuseles and the percentage of hemoglobin. The increase occurred earliest and was most rapid in physically fit men. Dallwig, Kolls and Loevenhart (3) observed that animals kept at normal atmospheric pressure but under low oxygen showed a definite increase in the blood counts at the time of the first observations, viz., after two or three days. Six rabbits living at 352 mm. Hg. pressure required as much as twenty-four to forty-eight hours for the increase to become definite.

All of the above observations were made after hours or even days of exposure to the effects of high altitudes and deficiency of oxygen. In order that the aviator may benefit by blood compensatory changes they would have to occur during exposures of thirty minutes to three hours. We have, therefore, investigated the blood changes that occur in men subjected to a lowered barometric pressure in a low pressure chamber and to low oxygen, 10 per cent, for intervals not exceeding two hours.

In a preliminary report by one of us (8) it was shown that in short exposures at least 25 per cent of all men examined had a well-defined increase in the percentage of hemoglobin. Corbett and Bazett (9) using a low oxygen method conclude that after about half an hour some degree of blood concentration may occur.

Blood for the estimation of hemoglobin was obtained in the usual manner by pricking the finger. In a few experiments with the Dreyer Nitrogen Apparatus it was taken from the lobe of the ear. In many cases the blood from the finger was compared with blood taken without stasis from a prominent vein in the forearm. Blood was obtained from the vein with a 10 cc. Record syringe and put in a short tube containing a little sodium oxalate. After a thorough stirring a few drops were taken with a pipette and put on a watch glass from which the sample was immediately taken into the blood pipette.

In the earlier experiments the Gower-Haldane carbon monoxide method (10) was used. Each sample was matched at once with the standard in the low pressure chamber by the aid of a white background and a "daylight" electric lamp. It was found more convenient, because of difficulty with the carbon monoxide supply, to dilute the blood in small test tubes containing 0.4 per cent ammonia. These samples were later transferred with proper rinsing to the Gower-Haldane graduated tube, saturated with carbon monoxide, and diluted further. The last sample taken in the experiment was always received directly in the Gower-Haldane tube.

Several experiments were carried out using the Palmer method (11). It was found difficult to keep the 1 per cent standard carbon monoxide blood, therefore the normal samples were usually considered as 100 per cent and the later samples were matched against them with the Duboseq colorimeter. The most convenient method was found to be a modification of the Palmer method which consisted in diluting the blood in 5 cc. of N/10 hydrochloric acid, instead of 0.4 per cent ammonia. The diluting fluid was measured in small test tubes which were stoppered with cotton and taken into the low pressure chamber. The blood was rinsed immediately into the diluting fluid from the 0.05 cc. pipette. The samples were matched at the end of the experiment as in the Palmer method.

Blood for the erythrocyte counts was taken by pricking the finger so that a free flow was obtained. The same Thoma mixing pipette was used for all comparative counts, and the blood was diluted in Hayem's solution. Two drops were put in a Levy double counting chamber with Neubauer ruling.

*Experiments in the low pressure chamber.* These experiments constitute the major part of this study. Throughout the work we adopted the plan of lowering the barometric pressure within the chamber at a rate that would be comparable to ascending in the air at the rate of 1000 feet per minute. The following altitudes were employed: 425, 395 and 380 mm. Hg., which are the pressures ordinarily encountered at 15,000, 17,000 and 18,000 feet respectively. The desired pressure having been attained it was then maintained for a time during which several samples of blood were taken and other observations made. From 30 to 100 minutes was the usual exposure to the lowered pressure.

A total of forty-five experiments was made upon thirty-five men, five of whom served as subjects two to five times. In fifteen of the experiments the hemoglobin determinations were made on blood from a vein of the arm as well as from the peripheral vessels of the finger. Also in fifteen other experiments the erythrocytes were counted as a check against the final hemoglobin determination. The data from these fifteen cases are given in table 2. The results for the thirty examinations in which the hemoglobin changes only were considered are collected in table 1.

The blood changes were definite in thirty-five, or in 78 per cent, of the experiments. In only ten, or 22 per cent, was the period of exposure to the lowered barometric pressure too brief or not sufficiently low to cause the blood response. Of the eight cases held at a pressure

TABLE 1  
*Low barometric pressure and hemoglobin*

| NAME AND DATE | MINUTES | HEMO-<br>GLOBIN | INCREASE<br>IN<br>PER CENT | NAME AND DATE    | MINUTES     | HEMO-<br>GLOBIN | INCREASE<br>IN<br>PER CENT |                  |     |     |
|---------------|---------|-----------------|----------------------------|------------------|-------------|-----------------|----------------------------|------------------|-----|-----|
| 15,000 feet   |         |                 | 4.3                        | 18,000 feet      |             |                 | 6.9                        |                  |     |     |
| A. F. H.      | 0       | 94              |                            | N. E. F.         | 0           | 94              |                            |                  |     |     |
| May 21        | 15      | 94              |                            | June 7,<br>1918  | 25          | 96              |                            |                  |     |     |
|               | 55      | 97              |                            |                  | 45          | 96              |                            |                  |     |     |
|               | 85      | 98              |                            |                  | 65          | 97              |                            |                  |     |     |
| G. W. D.      | 0       | 104             |                            | 83               | 100         | 0-V<br>85-V     |                            | 95               | 4.2 |     |
|               |         | 104             |                            | 99               |             |                 |                            |                  |     |     |
|               |         | 105             |                            |                  |             |                 |                            |                  |     |     |
|               |         | 106             |                            |                  |             |                 |                            |                  |     |     |
| May 23        | 15      | 104             |                            | R. S. S.         | 0           | 102             |                            | June 10,<br>1918 | 25  | 104 |
|               |         | 105             | 35                         |                  | 104         |                 |                            |                  |     |     |
|               |         | 106             | 50                         |                  | 106         |                 |                            |                  |     |     |
|               |         | 106             | 63                         |                  | 107         |                 |                            |                  |     |     |
| W. H. G.      | 0       | 96              | 71                         | 106              | 0-V<br>75-V | 102             | 3.9                        |                  |     |     |
|               |         | 103             |                            | 102              |             |                 |                            |                  |     |     |
|               |         | 106             |                            | 104              |             |                 |                            |                  |     |     |
|               |         | 105             |                            | 104              |             |                 |                            |                  |     |     |
|               |         | 105             |                            | 104              |             |                 |                            |                  |     |     |
| May 24        | 15      | 105             | 9.4                        | N. G. B.         | 0           | 107             | June 11,<br>1918           | 25               | 110 |     |
|               |         | 105             |                            |                  | 107         | 35              |                            | 110              |     |     |
|               |         | 105             |                            |                  | 107         | 50              |                            | 110              |     |     |
|               |         | 105             |                            |                  | 107         | 65              |                            | 112              |     |     |
| W. B. M.      | 0       | 104             | 5.8                        | 80               | 112         | 95              | 111                        | 100              | 112 | 4.7 |
|               |         | 102             |                            |                  | 111         |                 |                            |                  |     |     |
|               |         | 104             |                            |                  | 107         |                 |                            |                  |     |     |
|               |         | 104             |                            |                  | 107         |                 |                            |                  |     |     |
|               |         | 104             |                            |                  | 107         |                 |                            |                  |     |     |
|               |         | 110             |                            |                  | 107         |                 |                            |                  |     |     |
| May 27        | 15      | 106             | 0.0                        | W. B. M.         | 0           | 105             | June 12,<br>1918           | 27               | 105 |     |
|               |         | 106             |                            |                  | 105         | 50              |                            | 111              |     |     |
|               |         | 106             |                            |                  | 110         | 55              |                            | 110              |     |     |
|               |         | 107             |                            |                  | 105         | 0-V             |                            | 105              |     |     |
|               |         | 107             |                            |                  | 105         | 56-V            |                            | 109              |     |     |
|               |         | 106             |                            |                  | 105         | 109             |                            | 3.8              |     |     |
| W. O. K.      | 0       | 106             | 0.0                        | H. M. T.         | 0           | 112             | June 14,<br>1918           | 25               | 112 |     |
|               |         | 106             |                            |                  | 112         | 43              |                            | 114              |     |     |
|               |         | 106             |                            |                  | 114         | 60              |                            | 114              |     |     |
|               |         | 107             |                            |                  | 114         | 79              |                            | 114              |     |     |
|               |         | 107             |                            |                  | 108         | 0-V             |                            | 108              |     |     |
|               |         | 107             |                            |                  | 110         | 75-V            |                            | 110              |     |     |
| 17,000 feet   |         |                 | 2.2                        |                  |             |                 | 1.8                        |                  |     |     |
| F. S. V.      | 0       | 92              |                            | June 26,<br>1918 | 25          | 112             |                            |                  |     |     |
|               |         | 93              |                            |                  | 43          | 114             |                            |                  |     |     |
|               |         | 94              |                            |                  | 60          | 114             |                            |                  |     |     |
|               |         | 94              |                            |                  | 79          | 114             |                            |                  |     |     |
|               |         | 98              |                            |                  | 108         |                 |                            |                  |     |     |
| 0-V           | 98      | 2.0             |                            | 75-V             | 110         | 1.8             |                            |                  |     |     |
|               |         |                 |                            |                  |             |                 |                            | 100              |     |     |
|               |         |                 |                            |                  |             |                 |                            |                  |     |     |

TABLE 1—Continued

| NAME AND DATE | MINUTES | HEMO-GLOBIN | INCREASE IN PER CENT | NAME AND DATE | MINUTES | HEMO-GLOBIN | INCREASE IN PER CENT |
|---------------|---------|-------------|----------------------|---------------|---------|-------------|----------------------|
| B. M. L.      | 0       | 96          |                      | W. C. W.      | 0       | 98          |                      |
| June 17,      | 26      | 96          |                      | July 16,      | 64      | 102         |                      |
| 1918          | 40      | 99          |                      | 1918          | 78      | 104         | 6.1                  |
|               | 56      | 106         |                      |               |         |             |                      |
|               | 75      | 104         | 8.3                  | L. G. R.      | 0       | 100         |                      |
|               |         |             |                      | July 19,      | 40      | 100         |                      |
| A. W. L.      | 0       | 99          |                      | 1918          | 68      | 102         |                      |
| June 18,      | 26      | 100         |                      |               | 80      | 103         | 3.0                  |
| 1918          | 43      | 98          |                      |               |         |             |                      |
|               | 55      | 100         |                      | L. J. S.      | 0       | 108         |                      |
|               | 65      | 100         |                      | July 26,      | 45      | 108         |                      |
|               | 75      | 98          |                      | 1918          | 63      | 108         | 0.0                  |
|               | 85      | 100         | 0.0                  |               |         |             |                      |
|               | 0-V     | 98          |                      | E. W. B.      | 0       | 98          |                      |
|               | 86-V    | 100         | 2.3                  | June 30,      | 25      | 98          |                      |
|               |         |             |                      | 1918          | 40      | 98          |                      |
| L. F. M.      | 0       | 103         |                      |               | 60      | 100         |                      |
| July 21,      | 25      | 102         |                      |               | 80      | 102         |                      |
| 1918          | 35      | 102         |                      |               | 92      | 104         | 6.1                  |
|               | 45      | 104         |                      |               |         |             |                      |
|               | 55      | 107         |                      | I. M.         | 0       | 108         |                      |
|               | 65      | 106         | 3.0                  | July 1,       | 79      | 111         | 2.8                  |
|               | 0-V     | 104         |                      | 1918          | 0-V     | 107         |                      |
|               | 68-V    | 106         | 1.9                  |               | 80-V    | 110         | 2.8                  |
|               |         |             |                      |               |         |             |                      |
| G. C. W.      | 0       | 98          |                      | H. W. B.      | 0       | 106         |                      |
| June 25,      | 27      | 94          |                      | July 2,       | 58      | 110         | 3.8                  |
| 1918          | 41      | 94          |                      | 1918          | 0-V     | 108         |                      |
|               | 50      | 97          |                      |               | 60-V    | 112         | 3.7                  |
|               | 82      | 100         | 2.0                  |               |         |             |                      |
|               | 0-V     | 97          |                      | B. F.         | 0       | 106         |                      |
|               | 83-V    | 99          | 2.1                  | July 2,       | 50      | 106         |                      |
|               |         |             |                      | 1918          | 66      | 109         | 2.7                  |
| W. B. M.      | 0       | 104         |                      |               |         |             |                      |
| June 28,      | 25      | 103         |                      | D. T. R.      | 0       | 98          |                      |
| 1918          | 40      | 104         |                      | July 2,       | 40      | 97          |                      |
|               | 55      | 107         |                      | 1918          | 60      | 97          |                      |
|               | 72      | 110         | 5.8                  |               | 88      | 97          | 0.0                  |
|               | 0-V     | 106         |                      |               | 0-V     | 100         |                      |
|               | 75-V    | 108         | 1.9                  |               | 89-V    | 100         | 0.0                  |
|               |         |             |                      |               |         |             |                      |
| A. F. H.      | 0       | 94          |                      | H. J. M.      | 0       | 92          |                      |
| July 15,      | 40      | 95          |                      | July 5,       | 41      | 89          |                      |
| 1918          | 60      | 95          |                      | 1918          | 59      | 90          | 0.0                  |
|               | 78      | 98          | 4.3                  |               | 0-V     | 90          |                      |
|               |         |             |                      |               | 61-V    | 89          | 0.0                  |



TABLE 1—*Concluded*

| NAME AND DATE | MINUTES | HEMO-GLOBIN | INCREASE IN PER CENT | NAME AND DATE | MINUTES | HEMO-GLOBIN | INCREASE IN PER CENT |
|---------------|---------|-------------|----------------------|---------------|---------|-------------|----------------------|
| A. F. H.      | 0       | 99          | 5.0                  | E. C. S.      | 0       | 100         | 7.0                  |
| July 8,       | 40      | 100         |                      | April 29,     | 29      | 108         |                      |
| 1918          | 60      | 104         |                      | 1919          | 57      | 107         |                      |
|               | 86      | 104         |                      |               | 77      | 109         |                      |
|               |         |             |                      |               | 92      | 107         |                      |
| P. S. B.      | 0       | 104         | 4.8                  | N. E. B.      | 0       | 100         | 10.0                 |
| July 9,       | 36      | 102         |                      | 1919          | 42      | 108         |                      |
| 1918          | 60      | 110         |                      |               | 75      | 110         |                      |
|               | 85      | 109         |                      |               | 76      | 110         |                      |

V = Blood from vein.

corresponding to 15,000 feet three, or 38 per cent, failed to compensate, while among the five at 17,000 feet one, or 20 per cent, and among the thirty-two at 18,000 feet six, or 19 per cent, did not respond.

There were six men, 13 per cent, who showed a well-defined increase in the hemoglobin within the first 26 minutes of the experiment. This included the time allowed for ascent which varied from 15 to 18 minutes. The majority of men require between 45 and 60 minutes for the increase to be sufficient to be detected by the methods used.

There was a marked parallelism between the blood from the finger and blood from the vein, but in almost every case that from the vein was found to contain slightly less hemoglobin than that from the finger.

In the fifteen experiments in which the erythrocytes were counted there was an increase per cubic millimeter in each case in which the hemoglobin gave evidence of concentration. In five of these experiments no blood change occurred. The relation between the increase in the number of erythrocytes and hemoglobin shows a greater increase in erythrocytes than hemoglobin in seven of the ten positive cases.

W. B. M. served five times as a subject, once at 425 and four times at 380 mm. Hg. His hemoglobin percentage increases were 5.8, 4.8, 5.8, 0 and 8.8 respectively. A. F. H. was examined four times, one at 425 and three at 380 mm. respectively, with 4.3, 5, 4.3 and 0 per cent increases in hemoglobin. W. H. G. in two times showed 9.4 and 6.1 per cent, and B. R. L. in two experiments showed 8.4 and 8 per cent rises in hemoglobin. W. O. K. at 425 mm. failed to show a response, but at 395 mm. Hg. had an increase of 7 per cent in hemoglobin. The surprising feature of these repeated cases is the fact that, when a defi-

TABLE 2  
*Erythrocytes and hemoglobin in low pressure chamber*

| NAME     | DATE              | PRESSURE:<br>mm. | ALTITUDE<br>feet | LENGTH<br>OF<br>EXPERI-<br>MENT<br>minutes | HEMOGLOBIN |                   |                    | ERYTHROCYTES |                   |                    |
|----------|-------------------|------------------|------------------|--|------------|-------------------|--------------------|--------------|-------------------|--------------------|
|          |                   |                  |                  |  | Normal     | At end of<br>hold | Change<br>per cent | Normal       | At end of<br>hold | Change<br>per cent |
| F. C. P. | December 9, 1918  | 425              | 15,000           | 75   | 100        | 98                | 0.0                | 5,048,000    | 5,256,000         | 0.0                |
| T. S. G. | December 10, 1918 | 425              | 15,000           | 60   | 100        | 104               | 4.0                | 5,120,000    | 5,368,000         | 4.8                |
| C. P. C. | December 13, 1918 | 425              | 15,000           | 61   | 122        | 120               | 0.0                | 4,792,000    | 4,480,000         | 0.0                |
| J. H. N. | December 31, 1918 | 395              | 17,000           | 91   | 83         | 91                | 9.8                | 4,440,000    | 5,064,000         | 12.3               |
| K. O. W. | January 6, 1919   | 395              | 17,000           | 82   | 82         | 89                | 8.3                | 4,308,000    | 4,966,000         | 15.2               |
| F. D.    | January 13, 1919  | 395              | 17,000           | 86   | 91         | 91                | 0.0                | 5,200,000    | 4,983,000         | 0.0                |
| W. O. K. | January 14, 1919  | 395              | 17,000           | 102  | 86         | 92                | 7.0                | 4,868,000    | 5,375,000         | 10.4               |
| W. H. G. | July 10, 1918     | 380              | 18,000           | 102  | 98         | 104               | 6.1                | 5,216,000    | 5,720,000         | 9.5                |
| E. A. R. | July 13, 1918     | 380              | 18,000           | 83   | 95         | 98                | 3.2                | 5,232,000    | 6,080,000         | 16.0               |
| A. F. H. | July 30, 1918     | 380              | 18,000           | 69   | 99         | 100               | 0.0                | 5,080,000    | 5,012,000         | 0.0                |
| W. B. M. | July 31, 1918     | 380              | 18,000           | 56   | 104        | 103               | 0.0                | 4,936,000    | 4,704,000         | 0.0                |
| B. R. L. | August 5, 1918    | 380              | 18,000           | 145  | 107        | 116               | 8.4                | 4,672,000    | 5,600,000         | 20.0               |
| W. B. M. | December 27, 1918 | 380              | 18,000           | 85   | 91         | 99                | 8.8                | 4,752,000    | 4,931,000         | 3.8                |
| K. O. N. | April 30, 1919    | 380              | 18,000           | 83   | 100        | 106               | 6.0                | 4,774,000    | 5,520,000         | 13.7               |
| B. R. L. | May 12, 1919      | 380              | 18,000           | 80   | 100        | 108               | 8.0                | 5,274,000    | 5,732,000         | 8.1                |

The ascent was made at the rate of 1000 feet a minute.

nite response does occur, the total increase is so often approximately the same in an individual. Just why there was failure in the blood response in the two men who ordinarily reacted well to low oxygen is not indicated in our data. It is evident that there are physiological conditions under which an individual may not react in equal degree every time he encounters a given barometric pressure. We believe that when the blood fails to give the increase in red corpuscles and hemoglobin under the low oxygen of low barometric pressures that a heavier burden is thrown upon the respiration and the circulation of the blood.

*Experiments at normal atmospheric pressure and 10 per cent oxygen.* In these experiments the subject breathed atmospheric air diluted with nitrogen by the Dreyer Nitrogen Low Oxygen apparatus (12). Starting with undiluted air, 20.96 per cent oxygen, the subject breathing through a mask, the nitrogen was gradually added in greater and greater proportion so that by the end of 20 minutes the mixture contained only 10 per cent oxygen. Ten per cent oxygen at 760 mm. Hg. pressure corresponds in oxygen partial pressure to an altitude of approximately 19,000 feet. The subject of the experiment was held at this level of oxygen for from 30 to 90 minutes, thus he was kept under low oxygen for a period of from 50 to as much as 112 minutes.

Only seven men were examined for the hemoglobin changes by this method, four of them gave a positive response (see table 3). In three of the men the increase in hemoglobin had already begun when the first sample of blood was taken, 20 to 26 minutes, which was soon after 10 per cent oxygen was reached.

Two of the men, W. O. K. and E. A. R., were also tested in the low pressure chamber. W. O. K. showed an 8 per cent increase in hemoglobin under 10 per cent oxygen and 7 per cent under 395 mm. barometric pressure. E. A. R. with the low oxygen gave 5 per cent increase in hemoglobin and with the low pressure 380 mm. Hg., gave 3.2 per cent.

The results obtained by the two methods of subjecting men to low oxygen—lowered barometric pressure and lowered oxygen percentage—show that an increase in hemoglobin and the red corpuscles of the blood may result during short exposures. About 60 per cent of the men subjected to 10 per cent oxygen and 78 per cent of those subjected to low barometric pressure showed within from 15 to 90 minutes clearly defined increases in hemoglobin that ranged between 1.8 and 10 per cent. From the data presented it appears that the majority of men make the blood compensation rather quickly. Some delay is usually present in this response to low oxygen, but the lag is surprisingly short.

We have not investigated the mechanism by means of which these quick and early blood changes occur, when the organism is subjected to lowered partial pressure of oxygen. Views have differed as to the mechanism by which the marked increases in erythrocytes and hemo-

TABLE 3  
*Normal atmospheric pressure, oxygen 10 per cent*

| NAME AND DATE | MINUTES | HAEMO-<br>GLOBIN | INCREASE<br>IN PER<br>CENT | NAME AND DATE | MINUTES | HEMO-<br>GLOBIN | INCREASE<br>IN PER<br>CENT |
|---------------|---------|------------------|----------------------------|---------------|---------|-----------------|----------------------------|
| P. A.         | 0       | 98               |                            | C. A. C.      | 0       | 115             |                            |
| May 25,       | 20      | 101              |                            | June 1,       | 22      | 112             |                            |
| 1918          | 37      | 99               |                            | 1918          | 40      | 113             |                            |
|               | 57      | 99               | 0.0                        |               | 55      | 116             |                            |
|               |         |                  |                            |               | 65      | 114             |                            |
| G. B. H.      | 0       | 100              |                            |               | 75      | 114             |                            |
| May 28,       | 18      | 100              |                            |               | 90      | 116             |                            |
| 1918          | 34      | 100              |                            |               | 100     | 113             | 0.0                        |
|               | 46      | 100              |                            |               |         |                 |                            |
|               | 70      | 100              |                            | W. O. K.      | 0       | 100             |                            |
|               | 82      | 104              | 4.0                        | June 3,       | 26      | 102             |                            |
|               |         |                  |                            | 1918          | 39      | 106             |                            |
| C. N.         | 0       | 109              |                            |               | 50      | 108             | 8.0                        |
| May 29,       | 20      | 107              |                            |               |         |                 |                            |
| 1918          | 40      | 107              |                            | E. A. R.      | 0       | 100             |                            |
|               | 75      | 106              |                            | June 4,       | 20      | 102             |                            |
|               | 104     | 106              |                            | 1918          | 40      | 103             |                            |
|               | 112     | 106              | 0.0                        |               | 55      | 103             |                            |
|               |         |                  |                            |               | 74      | 105             |                            |
| W. A. B.      | 0       | 104              |                            |               | 81      | 105             | 5.0                        |
| May 31,       | 22      | 106              |                            |               | 0-V     | 90              |                            |
| 1918          | 41      | 106              |                            |               | 80-V    | 102             | 13.3                       |
|               | 56      | 108              |                            |               |         |                 |                            |
|               | 71      | 112              |                            |               |         |                 |                            |
|               | 81      | 112              |                            |               |         |                 |                            |
|               | 91      | 112              | 7.7                        |               |         |                 |                            |

V = Blood from vein.

globin occur during residence at a high altitude. Schneider and Havens (2) have given their opinion of the changes in the blood on adaptation to high altitudes as follows:

A rapid increase in the number of red corpuscles and percentage of hemo-globin in the blood of the peripheral vessels occurs during the first two to four days of residence at the high altitude, then follows a more gradual increase for



about three weeks. The initial rapid increase is brought about in part by throwing into the systemic circulation a large number of red corpuscles that under ordinary circumstances at low altitudes are side-tracked and inactive, and in part by a concentration resulting from a loss of fluid in the blood. The more gradual increase in red corpuscles and hemoglobin extending over several weeks is brought about by the increased activity of the blood-forming centers so that there results a large increase in the total number of corpuscles and amount of hemoglobin.

The question which presents itself here is whether the early increase in hemoglobin and red corpuscles, such as we obtained within the short space of an hour, is to be attributed to concentration of the blood, i.e., a reduction in the total blood volume, or to changes in the distribution of the erythrocytes. A suddenly increased production of hemoglobin and erythrocytes by the bone marrow is improbable. That the increase is not caused by an increased evaporation of water from the body is indicated by the conditions of experimentation and the fact that in some men the change is already well developed within a 15 to 20 minute period and that perspiration is not noticeably increased. It has been claimed that all aviators engaged on long patrols at 17,000 feet, or over, complain of over-filling of the bladder. Birley (13) looks upon this as confirmatory of the theory that one factor in the reaction of the organism to lowered barometric pressure is a concentration of the blood at the expense of the plasma. Against this theory of a polyuria we urge that the time, 15 to 20 minutes as seen in a few cases, is too short. If this were the mechanism, all subjects should be conscious of the filling of the bladder. The majority of our subjects have not been conscious of an increased action of the kidneys. In fact we are inclined to believe that only the nervousness experienced during the first time or so spent in the low pressure chamber gives rise to the sensation of an increased sensation of urine. Several observations made, in and out of the low pressure chamber in this laboratory, on the secretion of urine fail to confirm the polyuria theory.

The increased production of urine during flights at high altitudes finds an explanation in the action of cold. Against the likelihood of this increased urine formation being evidenced in blood concentration we have the studies of Bogert, Underhill and Mendel (15) in which they introduced large volumes of fluids without appreciably affecting the unit blood content of hemoglobin.

Another possibility is that lowered oxygen tension changes the property of the muscles so that they absorb a larger volume of water and

in sufficient quantity to reduce the blood volume. We know of no experimental proof for this view.

The percentage of increase in hemoglobin and erythrocytes observed in these short exposures to low oxygen is within the limits of those observed after various forms of physical exertion. Schneider and Havens (14) found that exercise increased the hemoglobin to from 3.5 to 11 per cent and the number of red corpuscles per cubic millimeter to from 3.2 to 22 per cent. They held that this increase was the result of throwing into the systemic circulation a large number of erythrocytes that under ordinary circumstances are side-tracked and inactive. The same explanation might be advanced for the low oxygen compensatory blood changes. A decision as to the value of the concentration theory and the theory of the dormant supply of erythrocytes cannot be made at this time.

The physiological significance of an increase in erythrocytes and hemoglobin during exposure to low oxygen is that a unit volume of blood can carry for a given oxygen pressure more oxygen than normally. The supposition is that the aviator whose blood concentrates will, other things being equal, tolerate high altitudes more comfortably and more efficiently than the man who does not react with an increase in erythrocytes and hemoglobin.

#### SUMMARY

1. Low oxygen tension was produced by lowering the barometric pressure in different experiments to 380, 395 and 425 mm. Hg., and by replacing oxygen by nitrogen gradually until 10 per cent oxygen was reached. The subjects were maintained at the low oxygen tensions from periods varying from 30 minutes to 145 minutes.

2. Blood for the estimation of hemoglobin was taken from a finger and a vein. The determinations were made by the Gower-Haldane carbon monoxide method, by the Palmer method, and by a modified Palmer method using hydrochloric acid.

3. An increase in hemoglobin was obtained under reduced barometric pressure in 78 per cent of all examinations made. The majority of the men required between 40 and 60 minutes for the increase to become definite, 13 per cent showed a well defined increase within 26 minutes. In the experiments with 10 per cent oxygen 57 per cent gave the increase in hemoglobin.

4. In fifteen cases in which the erythrocytes and hemoglobin were determined corresponding changes occurred in both, 66 per cent were positive. The erythrocyte increase ranged between 3.8 and 20 per cent, the hemoglobin between 3.2 and 9.8 per cent.

5. In the several experiments on the same individual, the increase in the hemoglobin was approximately the same each time.

6. The blood concentration theory and the theory of the dormant supply of erythrocytes are briefly contrasted.

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## CIRCULATORY RESPONSES TO LOW OXYGEN TENSIONS

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It is a well established fact that residence at high altitudes exerts a profound influence on the human body. Because of the recent development of aviation, which has made rapid ascents to very high altitudes possible, the knowledge of the effects of short exposures to the influence of altitude assumes practical importance. The adaptive reactions to altitude observed in men who take up residence at a high altitude develop rather slowly and are of a fairly permanent character (1). The aviator does not remain long enough at a high altitude to benefit from slow adaptive physiological changes. If he tolerates and does well, he must depend upon rapid compensatory changes to provide the oxygen needed by the tissues. That the body is capable of responding to abrupt and great changes in atmospheric pressure has been proved by studies made in this laboratory (2). Among the physiological responses made to low oxygen tensions are those of the circulatory mechanism. The object of this paper is to present observations on the pulse rate and the arterial blood pressures made upon men who were subjected to low oxygen tension produced in three ways, by low barometric pressures in a low pressure chamber, by low percentage of oxygen caused by rebreathing under normal atmospheric pressure, and by diluting the respired air with increasing amounts of nitrogen.

The low pressure chamber and its control has been briefly described elsewhere (3). The rebreathing experiments were made with the Henderson-Pierce rebreathing machine (3). The Dreyer method (4) was used to dilute atmospheric air with nitrogen which was delivered to the subject by means of an American model of a Tissot gas mask.

Two types of experiments have been carried on. In one the oxygen tension was gradually reduced until the mental condition of the subject showed that he was no longer able to compensate, or until syncope appeared. In the other group of experiments the oxygen tension



was reduced at a rate corresponding to an ascent of 1000 feet per minute until a desired level,—that is, 15,000, 17,000, 18,000 feet,—was reached, after which the level was maintained for from 30 to 90 or more minutes.

Throughout all experiments the subjects were seated, and it was the rule to count the pulse rate for half a minute during each minute of the experiment. Usually the count was begun at 45 on the seconds dial of a stop-watch and continued to 15 and then recorded as though taken on the minute. In the remaining portion of the minute the blood pressures were taken. To keep such a record requires close attention and extensive experience. During each experiment one man was held responsible for all these determinations and was relieved of the necessity of watching the condition of the subject and arrangements of experimentation.

The arterial blood pressures were determined by the auscultatory method with the aid of a Tycos sphygmomanometer which was adjusted over the brachial artery of the left arm. A Bowles stethoscope with special arm band was used. The systolic pressure was read at the beginning of the first phase and the diastolic pressure was measured at the fourth phase, that is, at the dulling of the intense sounds of the third phase. In many of the low pressure chamber experiments, in which the pump was run continuously, the diastolic determinations had to be omitted because of noise.

In the selection of subjects an effort was made to secure men who had not been doing physical work during the hour previous to the experiment. Before any observations were made the subject was allowed to sit quietly for a while, after which a number of preliminary determinations of the pulse rate and arterial blood pressures were made to establish the so-called normal. Occasionally the first time a subject appeared for the low oxygen test he showed some degree of anxiety or excitement in a slightly rapid pulse or increased systolic pressure. After a few moments of tactful conversation this nervousness was usually overcome. In much of our work we have used men accustomed to being subjects, and in these the excitement effect is often absent, or if present almost negligible. In all experiments in which an attempt is made to detect the earliest effects of low oxygen, this psychic factor has to be considered. Unquestionably it often masks the onset of heart rate acceleration due to low oxygen.

*Earlier work on heart rate during exposures to low oxygen tension.* When the oxygen tension of the respired air is decreased, the blood for

a time may be less completely saturated with oxygen than when air of normal composition and pressure is breathed. During such a condition the tissues would very likely be inadequately supplied with oxygen. If during this period the blood contains less oxygen than normally, and the rate of blood flow through the capillaries is increased, the tissues will be provided with the oxygen demanded for their activity. More blood flowing to the tissues, even though it contains a lessened amount of oxygen, results to some extent in maintaining the oxygen tension in the tissues.

Throughout our experimental work with low oxygen we have assumed that an increase in the rate of the heart beat, the arterial pressures being maintained within normal limits, meant an increase in the per-minute output of the heart. Under ordinary circumstances an increase in the pulse rate during exercise is recognized as satisfactory evidence that the output of the heart has increased and the flow of the blood has accelerated.

The idea that an increase in the heart rate is a method of compensating for lack of oxygen is by no means new. Finkler (5) in 1875 induced anemia in dogs by bleeding and found that the decrease in the oxygen content of the blood may stimulate both the heart and respiration to greater activity. These, as Lusk (6) has pointed out, are efforts of compensation for the decrease in oxygen, although nothing resembling asphyxia is present. Kohler (7) in 1877 interfered with the respiration of rabbits by compressing the trachea by means of a lead wire tied around it. This was followed by compensation by means of increased respiration and heart activity so that there was no lack of oxygen in the animals. In these animals the heart hypertrophied.

Experimental studies on men living at a high altitude have seemed to prove an increased rate of blood flow. Schneider and Sisco (8), on Pike's Peak by use of Stewart's hand-colorimeters, concluded that "the rate of blood flow in the hands of six men examined was increased . . . by an amount varying from 30 to 70 per cent." The increase in the rate of flow has been associated in part with an augmented rate of heart beat and a fall in the venous pressure, also in part with a dilatation of the arterioles. Kuhn (9) working on Monte Rosa demonstrated by calculations made from determinations of the oxygen capacity of the blood, the total oxygen consumption, and the pulse rate, that the heart rate responds to the influence of lowered barometric pressure by increasing its output per minute.

Hasselbach and Lindhard (10) working with three men in a pneumatic chamber failed to prove an increased blood flow with the nitrous-oxide method. It should be noted, however, that their pressure changes were too small to produce profound change. They maintained pressures of 589 to 514 mm. Hg. (6800 to 10,400 feet) for five to seven days and attained these pressures very slowly. Under these conditions other compensatory changes might have been sufficient to meet the call for oxygen.

Our knowledge of the influence of high altitudes on circulation has been secured chiefly from men living at high altitudes on mountains. Of all the circulatory changes due to diminished barometric pressure, the acceleration of the heart rate has been most studied. Mountain ascents, even when made passively by railway car or automobile are slow, 8000 feet in an hour and a half or longer, when compared with altitude flights in an aeroplane. It has been shown by studies on Pike's Peak (11), (14,110 feet), that the pulse rate does not accelerate immediately on arrival at the summit. It accelerates gradually in those who ascend passively by train and remain well, and requires several days to reach the maximum rates. In men who become mountain or altitude sick the augmentation comes on earlier and is greater than in those who remain well. Later the rate returns to the normal for the particular altitude. In men fatigued by walking to the summit the high altitude heart rate is usually established within a few hours.

*Changes in heart rate during a gradual decrease in oxygen tension.* Seventeen men served as subjects for a series of examinations in which the action of a steadily decreasing barometric pressure was compared with that of a steady decrease in the oxygen percentage by the rebreathing method. In this work it was customary to give the rebreathing test first, and on a later day the low pressure test. From the rebreathing data, in which the final oxygen percentage and the duration of the test in minutes were recorded, we calculated the barometric pressure that was equivalent in oxygen tension to the final rebreathing oxygen per cent, and then determined the rate at which the pressure should be lowered in the low pressure chamber. It was thus possible to reproduce with a fair degree of accuracy in the low pressure chamber test the oxygen tension changes experienced during rebreathing examination. In two pairs of experiments (see G. F. H. and F. D., table 1) the rebreathing test was prolonged by introducing into the tank of the apparatus a continuous flow of oxygen. This, in effect, made the altitude ascent about three times slower than that of the average test. In about 70 per cent of the tests the men were carried down in oxygen



until they became inefficient as judged by the psychologist or the failure of the compensatory mechanism.

The response of the heart during rebreathing tests of 25 to 30 minutes duration has been described by Schneider (12). He reported that in men under a gradually decreasing oxygen supply the heart rate soon began to accelerate, at first by a slight increase of from one to three beats, and later by a very marked acceleration when the oxygen had fallen to between 13 and 9 per cent. The heart rate was shown to accelerate in a few men as early as 17.5 per cent of oxygen (5000 feet) while 12 per cent of all cases examined began to respond between 15.5 and 14.9 per cent oxygen, (8000 to 9000 feet).

In our series of low pressure and low oxygen percentage comparisons the similarity of the circulatory responses made by the individual to the two conditions was most striking. The data have been analyzed and tabulated in table 1. It was impossible to have the subject in exactly the same condition for each test because the two types of tests were separated by at least 24 hours and in one case by 18 days. Furthermore the degree of apprehension in the subjects differed for the tests. Some men dreaded going into the low pressure chamber and others disliked the mouthpiece and nose clip of the rebreather. The apprehension was of course registered in a quickened pulse rate or increased systolic pressure when the normals of each were compared with the determinations made at the start of the actual experiment.

When the psychic factor was not in evidence, the pulse rate, in each of the two kinds of low oxygen experiments, maintained for a short time the normal or pre-experiment rate and then gradually began to accelerate. In the majority of cases there was at first a slow increase in rate, but when the oxygen tension had fallen to that corresponding to from 15 to 10 per cent oxygen at normal atmospheric pressure, it gave way to a more rapid rate of acceleration.

The beginning of a pulse rate acceleration has been determined as to time and pressure, or oxygen per cent. Three of the men showed in both the low pressure chamber and under low oxygen per cent a definite gradual acceleration which began with the first change in pressure or oxygen per cent at the beginning of the experiment. In the seventeen comparisons the latest onset in the acceleration occurred at 15.2 per cent oxygen (8400 feet). We believe that the evidence proves the heart to be responsive to a slight decrease in oxygen in the air respired if the psychic acceleration is satisfactorily eliminated, as it has been in a great many of our cases. Fourteen times in this series of



comparisons, or in 41 per cent of the tests, the acceleration began between 17 and 15 per cent oxygen, that is, between altitudes of 5800 and 8800 feet. All men in this series showed an increase in heart rate before an oxygen percentage corresponding to 9000 feet was reached. Omitting from our calculations the cases that gave an immediate acceleration there were nine cases, or 26 per cent, that responded with an increase in heart rate at 18 or more per cent of oxygen (4000 feet or less).

It is generally found that men living at moderately high altitudes, 6000 to 9500 feet, to which they are acclimated, do not show an augmentation in the rate of heart beat. The reaction shown in our tests is an immediate compensation to low oxygen, and as will be seen later is not necessarily a permanent change which would be maintained so long as the particular oxygen tension was held.

The normal pulse rates and the maximum rates, which occurred when the oxygen tension was lowest, are recorded in table 1. In several of the runs the final heart rate was only 16 or 18 beats per minute above the preliminary or normal rate. In other men the acceleration was 45 and in one case 57 beats per minute. The smaller acceleration usually occurred in men who reached only 9 or 10 per cent oxygen, while the greater increase occurred with 6 and 7 per cent oxygen. The percentage of acceleration brings out more clearly the comparative differences; thus R. M. B. at 10 per cent oxygen had an acceleration of 19 per cent, and G. F. H. at 6.3 per cent oxygen showed a 79.3 per cent increase.

Those who would account for the circulatory change at reduced atmospheric pressure apart from decreased oxygen tension, would find it difficult to explain the parallelism observed in the responses of the heart to the two methods of experimentation employed in these pairs of tests, under low barometric pressure and low oxygen caused by rebreathing at normal atmospheric pressure. The heart rate responded at so nearly the same percentage of oxygen in eleven of the seventeen pairs of experiments that we are justified in speaking of them as duplicate responses. In F. D. the acceleration began at 17 and 16.9 per cent, in D. T. R. at 15.5 and 15.8 per cent, in R. M. B. at 15.4 and 16 per cent, in C. N. at 15.7 and 15.5 per cent and in G. M. at 19.2 and 20 per cent for the low pressure chamber and the rebreathing experiments respectively. The plotted curves (see fig. 1) showing the relationship between low pressure and low oxygen changes likewise indicate that the same cause must be operating in the two methods of experimentation.

TABLE 1  
*Comparison of rebreathing and low pressure chamber experiments. Oxygen tension reduced gradually until the end*

| NAME         | DATE    | REBREATH-<br>ING OR L. P.<br>CHAMBER | LENGTH<br>IN<br>MINUTES | FINAL OXYGEN                  |         | PULSE |     |                      | BEGINNING OF ACCELERATION |                               |                           |
|--------------|---------|--------------------------------------|-------------------------|-------------------------------|---------|-------|-----|----------------------|---------------------------|-------------------------------|---------------------------|
|              |         |                                      |                         | Per cent<br>or barom-<br>eter | Tension | Begin | End | Per cent<br>increase | Minute                    | Per cent<br>or barom-<br>eter | Tension<br>O <sub>2</sub> |
| G. F. H..... | 3/ 4/18 | Rebr.                                | 85                      | 8.5                           | 60.8    | 73    | 118 | 61.6                 | 12                        | 19                            | 145                       |
|              | 3/ 6/18 | L. P. C.                             | 85                      | 330                           | 69.1    | 66    | 98  | 48.5                 | 31                        | 600                           | 126                       |
| G. F. H..... | 3/ 5/18 | Rebr.                                | 24                      | 6.3                           | 47.9    | 72    | 129 | 79.3                 | 8                         | 16.3                          | 124                       |
|              | 3/ 8/18 | L. P. C.                             | 25                      | 325                           | 67.1    | 69    | 111 | 60.9                 | 2                         | 690                           | 145                       |
| S. I.....    | 3/ 4/18 | Rebr.                                | 31                      | 9.3                           | 70.6    | 82    | 111 | 35.4                 | At once                   |                               |                           |
|              | 3/ 8/18 | L. P. C.                             | 35                      | 338                           | 70.8    | 86    | 120 | 39.5                 | At once                   |                               |                           |
| F. D.....    | 3/ 5/18 | Rebr.                                | 36                      | 7.3                           | 55.5    | 84    | 126 | 50.0                 | 12                        | 17                            | 129                       |
|              | 3/ 4/18 | L. P. C.                             | 32                      | 365                           | 76.5    | 70    | 108 | 54.4                 | 4                         | 610                           | 128                       |
| F. D.....    | 3/ 6/18 | Rebr.                                | 90                      | 8.0                           | 60.8    | 80    | 117 | 46.2                 | 19                        | 18                            | 137                       |
|              | 3/ 7/18 | L. P. C.                             | 90                      | 310                           | 64.9    | 84    | 122 | 45.2                 | 40                        | 550                           | 115                       |
| C. H.....    | 4/ 9/18 | Rebr.                                | 26.5                    | 7.7                           | 58.5    | 92    | 123 | 33.7                 | At once                   |                               |                           |
|              | 4/12/18 | L. P. C.                             | 27                      | 282                           | 59.0    | 84    | 112 | 33.3                 | 9                         | 610                           | 128                       |
| C. K. R..... | 4/ 9/18 | Rebr.                                | 26.5                    | 8.2                           | 62.4    | 88    | 123 | 39.8                 | At once                   |                               |                           |
|              | 4/13/18 | L. P. C.                             | 27.0                    | 295                           | 61.8    | 96    | 123 | 28.1                 | At once                   |                               |                           |
| D. T. R..... | 4/20/18 | Rebr.                                | 27.5                    | 7.8                           | 59.4    | 81    | 114 | 40.7                 | 12                        | 15.5                          | 118                       |
|              | 4/24/18 | L. P. C.                             | 27.0                    | 308                           | 64.5    | 81    | 114 | 40.7                 | 12                        | 570                           | 119                       |

|              |   |         |          |      |      |      |    |     |      |         |     |
|--------------|---|---------|----------|------|------|------|----|-----|------|---------|-----|
| R. B.....    | { | 4/ 4/18 | Rebr.    | 25.5 | 8.6  | 65.4 | 78 | 123 | 57.7 | At once |     |
|              |   | 4/22/18 | L. P. C. | 27.0 | 310  | 64.9 | 74 | 112 | 51.4 | At once |     |
| G. W. D..... | { | 4/10/18 | Rebr.    | 26.5 | 6.9  | 52.5 | 80 | 114 | 42.5 | At once |     |
|              |   | 4/15/18 | L. P. C. | 25   | 300  | 62.8 | 87 | 105 | 20.7 | At once |     |
| I. M.....    | { | 4/10/18 | Rebr.    | 25   | 9.7  | 73.6 | 64 | 80  | 25.0 | 6       | 139 |
|              |   | 4/17/18 | L. P. C. | 25   | 360  | 75.5 | 64 | 92  | 43.8 | At once |     |
| R. M. B..... | { | 4/17/18 | Rebr.    | 25   | 10.0 | 76.0 | 84 | 100 | 19.0 | 11      | 122 |
|              |   | 4/19/18 | L. P. C. | 25   | 360  | 75.5 | 78 | 99  | 26.9 | 12      | 117 |
| A. M. J..... | { | 4/30/18 | Rebr.    | 25   | 9.2  | 70.0 | 90 | 108 | 20.0 | 10      | 124 |
|              |   | 5/ 1/18 | L. P. C. | 25   | 325  | 67.1 | 84 | 108 | 28.5 | 3       | 148 |
| F. L. D..... | { | 4/12/18 | Rebr.    | 20   | 9.3  | 70.6 | 64 | 90  | 40.5 | 14      | 137 |
|              |   | 4/16/18 | L. P. C. | 20   | 335  | 70.2 | 72 | 90  | 25.0 | 8       | 124 |
| C. N.....    | { | 4/15/18 | Rebr.    | 22   | 9.8  | 74.5 | 64 | 86  | 34.4 | 11      | 118 |
|              |   | 4/25/18 | L. P. C. | 22   | 345  | 72.2 | 58 | 76  | 31.1 | 11      | 117 |
| L. S. L..... | { | 4/11/18 | Rebr.    | 28   | 9.5  | 72.2 | 60 | 82  | 36.7 | 6       | 141 |
|              |   | 4/13/18 | L. P. C. | 28   | 350  | 73.4 | 59 | 84  | 42.4 | At once |     |
| G. M.....    | { | 4/23/18 | Rebr.    | 25   | 10.0 | 76.0 | 66 | 90  | 36.4 | 2       | 152 |
|              |   | 4/29/18 | L. P. C. | 25   | 360  | 75.5 | 66 | 90  | 36.4 | 4       | 146 |

The difference between the normal and maximum rates expressed in percentage increase also shows that the pulse response took place in about equal degree when equal oxygen tensions were reached. The discrepancies for G. F. H., 1 and 2, and G. W. D. in table 1 are explained by failure to reach the same lower limit in each experiment. The wide difference shown by I. M. and F. L. D. cannot be explained by our data. The parallelism in response is best shown by C. H., 33.7 and 33.3 per cent, C. N., 31.1 and 34.4 per cent, and G. M. with a 36.4 per cent total acceleration in the low pressure and low oxygen by the re-breathing method. The difficulty in reproducing exactly conditions

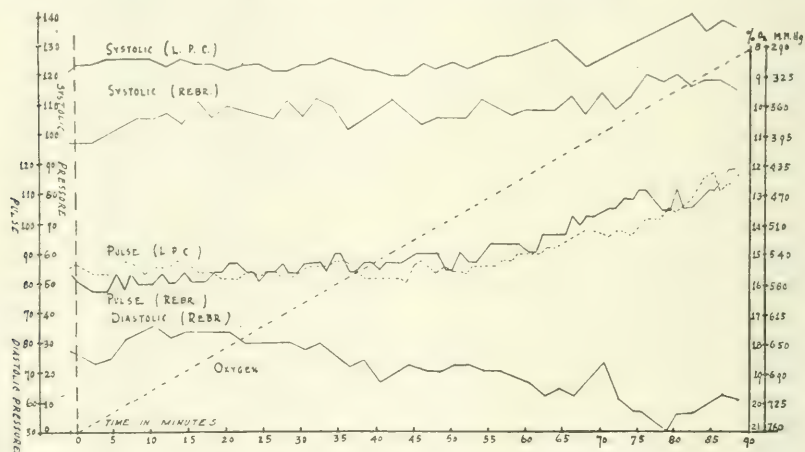


Fig. 1. F. D. Comparison of the rebreathing and low pressure chamber methods. Blood pressures and pulse rate taken every minute. This case illustrates the close correspondence in pulse response, although the experiments were made on different days. See table 1.

in experimentation and securing subjects who are exactly the same physically on two different days makes the parallelism here reported all the more striking. It is evidence for the theory that lack of oxygen, or decrease in oxygen tension, is the cause of the heart rate response under the two very different procedures.

Two sets of comparative experiments were conducted on G. F. H., and F. D. (see table 1), one of moderate length for each, and one extending over 85 and 90 minutes. In each set the oxygen and the pressure were gradually decreased throughout the period of experimentation. Rebreathing experiments carried on in this laboratory have shown that



when the oxygen is lowered rapidly, the subject compensates to a lower percentage than is possible when the rate of decrease in the oxygen is slower. The following cases illustrate the point. G. F. H. in a rebreathing experiment of 24 minutes compensated to 6.3 oxygen, but in one of 85 minutes he reached only 8.5 per cent. F. D. in 36 minutes compensated to 7.3 per cent, and in 90 minutes to 8 per cent oxygen. Unfortunately the low pressure chamber experiments for each were terminated for other reasons than failure of physiological compensations.

The data obtained from seven comparative experiments with the Dreyer nitrogen dilution method of giving low oxygen and the rebreathing method are given in table 2. Unfortunately in this series the attempt was not made to reproduce exactly in rate and low percentage the conditions of low oxygen experienced in rebreathing by the subject. The two examinations were never made on a man during the same day. The air breathed was under normal atmospheric pressure. In both we deal with a gradual decrease in oxygen percentage, that is to say, partial pressure of oxygen. The results show the similarity that was to be expected. The total acceleration and the plotted curves of the gradual increase in pulse rate corresponded in all of the comparisons made. The comparative sets of experiments on R. S. S., J. B. H., L. S. L. and P. S. B. show a very satisfactory parallelism. The onset of the acceleration was delayed longer in several of the experiments of this series than in any of the low pressure chamber series. P. S. B. in both the Dreyer and the rebreathing method showed no heart rate response until 11.3 per cent oxygen was reached, approximately 16,000 feet.

*Changes in the heart rate while a low oxygen level is maintained.* The majority of these experiments were conducted in the low pressure chamber. The barometric pressure was lowered to 425, 395 or 380 mm. Hg. (15,000, 17,000 and 18,000 feet) at the rate of 1000 feet per minute and held at that pressure for periods varying from 30 to 130 minutes, the pulse rate being taken every minute during the entire period. We selected these pressures because most men would stand them without discomfort or noticeable loss in efficiency.

In fifty cases the average pulse rate at 760 mm. was 74 per minute which points to a lack of anxiety in the subjects. The maximum rate for the men taken to 425 mm. (15,000 feet) was 89, and for 40 men at 395 and 380 mm. it was 94. The increase in rate at 425 mm. Hg. ranged between 5 and 19 beats. The percentage of acceleration ranged between 5.8 and 30. The average percentage acceleration in rate was

TABLE 2  
*Comparison of the rebreathing and diluted nitrogen experiments. Oxygen tension reduced gradually until the end*

| NAME          | DATE                    | REBREATH-<br>ING DILUTED<br>NITROGEN | LENGTH<br>IN<br>MINUTES | FINAL OXYGEN |         | PULSE |     |                      | BEGINNING OF ACCELERATION |                            |                           |
|---------------|-------------------------|--------------------------------------|-------------------------|--------------|---------|-------|-----|----------------------|---------------------------|----------------------------|---------------------------|
|               |                         |                                      |                         | Per cent     | Tension | Begin | End | Per cent<br>increase | Minute                    | Per cent<br>O <sub>2</sub> | Tension<br>O <sub>2</sub> |
| I. M. ....    | {<br>3/23/18<br>5/11/18 | Rebr.<br>Dil. N.                     | {<br>28.5<br>30.0       | 7.6          | 57.8    | 78    | 117 | 50.0                 | 6                         | 18.0                       | 137                       |
|               |                         |                                      |                         | 6.0          | 45.6    | 78    | 110 | 41.0                 | 20                        | 11.3                       | 86                        |
| R. S. S. .... | {<br>5/21/18<br>5/24/18 | Dil. N.<br>Rebr.                     | {<br>29.0<br>29.3       | 9.1          | 69.1    | 72    | 96  | 33.4                 | At once                   | 19.5                       | 148                       |
|               |                         |                                      |                         | 9.1          | 69.1    | 70    | 94  | 34.3                 | 4                         |                            |                           |
| J. B. H. .... | {<br>5/17/18<br>5/21/18 | Rebr.<br>Dil. N.                     | {<br>28.0<br>29.0       | 7.8          | 59.2    | 78    | 93  | 19.2                 | 15                        | 13.6                       | 103                       |
|               |                         |                                      |                         | 7.6          | 57.8    | 72    | 87  | 20.8                 | 22                        | 11.0                       | 84                        |
| L. S. L. .... | {<br>3/18/18<br>5/21/18 | Rebr.<br>Dil. N.                     | {<br>25.0<br>28.0       | 8.8          | 66.9    | 68    | 94  | 37.3                 | 4                         | 19.0                       | 144                       |
|               |                         |                                      |                         | 8.6          | 66.2    | 69    | 84  | 40.6                 | 22                        | 13.5                       | 103                       |
| C. F. W. .... | {<br>5/6/18<br>5/13/18  | Rebr.<br>Dil. N.                     | {<br>28.0<br>32.0       | 6.8          | 51.6    | 88    | 116 | 31.8                 | 14                        | 13.8                       | 105                       |
|               |                         |                                      |                         | 5.5          | 41.8    | 76    | 104 | 36.8                 | 16                        | 13.4                       | 102                       |
| A. L. D. .... | {<br>5/25/18<br>5/24/18 | Dil. N.<br>Rebr.                     | {<br>28.0<br>26.5       | 6.6          | 50.2    | 86    | 114 | 32.5                 | 14                        | 14.5                       | 110                       |
|               |                         |                                      |                         | 9.3          | 70.7    | 88    | 128 | 45.5                 | *                         |                            |                           |
| P. S. B. .... | {<br>5/6/18             | Rebr.<br>Dil. N.                     | {<br>28.0<br>30.5       | 7.7          | 58.5    | 80    | 100 | 20.0                 | 20                        | 11.3                       | 86                        |
|               |                         |                                      |                         | 6.5          | 49.4    | 78    | 102 | 23.7                 | 21                        | 11.3                       | 86                        |

\* Obscured by psychic rise.

18.7. The increase in rate at 380 mm. (18,000 feet) ranged between 6 and 45 beats. The percentage augmentation varied between 6.7 and 59, with an average increase in rate of 26.3 per cent above the normal at 760 mm.

The maximum pulse rate usually did not occur simultaneously with the arrival of the desired altitude. The lag was somewhat longer in the men taken to 380 mm., than in those at the lower altitude. In two cases in which the pressure was only 425 mm., the maximum rate was attained during the ascent. In the other men held at this level the lag varied between 2 and 7 minutes. The average lag at this pressure

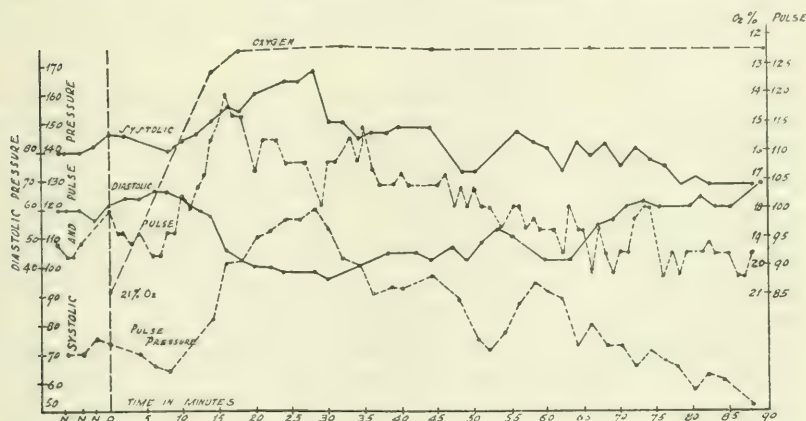


Fig. 2. K. D. Taken by the rebreathing method to 12.5 per cent oxygen (13800 feet) in 18 minutes and maintained at that level. Blood pressures and pulse rate taken every minute. This chart illustrates the return of the circulatory factors, while the low oxygen is being maintained, toward their original values. See table 5.

was 2.8 minutes. In all of the experiments in which a pressure of 380 mm. was reached and then maintained the average delay in the appearance of the maximum pulse rate was 6.6 per minutes. Every man showed some lag. In two the delay was only for 1 minute, but in several it was as long as 20 minutes, and in one the heart rate continued to increase gradually for 26 minutes, accelerating during this period from a rate of 90 to one of 108 beats per minute.

We also carried nine men by the Dreyer method to 10 per cent oxygen (19,400 feet) in 20 minutes and then held them at that level. In these cases a definite lag in the pulse rate in reaching its maximum

was observed. The shortest time taken to reach the maximum after the oxygen level was established was 3 minutes, the longest 38 minutes. The average lag was 14 minutes. These results compared with those obtained in the low pressure chamber seem to indicate that the lower the partial pressure in the air respired the slower will be the pulse in reaching its maximum rate.

Observations on the development of cyanosis are suggestive of the fact that the available oxygen is gradually decreasing within the blood during this period in pulse lag. Some cyanosis has been observed during the holding period for each level studied, but it was most conspicuous in the low pressure chamber at 380 mm. Hg. (18,000 feet). The cyanosis comes on slowly and, like the pulse rate, requires some minutes to reach its greatest degree.

In tables 3 and 4 have been tabulated the circulatory data obtained in forty experiments in the low pressure chamber in which the pressures were gradually reduced and then maintained at some time at 425, 395 and 380 mm. Hg. These data show that in many men the pulse rate does not maintain its maximum during the holding period at low pressure. Corbett and Bazett (13) on subjecting men to a constant per cent of low oxygen, observed for the pulse rate that "as adaptation takes place it tends to fall to a slightly lower level."

We find three types of pulse rate reaction during the holding period. These are: *a*, a definite and gradual decrease in rate after a brief period of maintained maximum; *b*, maintenance of the maximum rate; and *c*, a steady but slow rise in rate throughout the entire holding period. Our forty are distributed as follows: Type *a*, 29; *b*, 9; and *c*, 2. The amount of fall that occurs with adaptation is an individual matter. In some it is slight, only two to four beats, but in others the rate may return very nearly to normal. Other methods of subjecting to low oxygen gave similar results. During the holding period at 10 per cent oxygen with the Dreyer method nine men (see table 5) reacted as follows: five had a gradual retardation after maintaining the maximum pulse rate for a short time, three maintained the maximum rate and one gave a steady rise to the end.

In one long experiment with the rebreathing apparatus after the oxygen had been reduced to 13 per cent (12,400 feet) in 18 minutes, this level was maintained for 60 minutes by admitting pure oxygen into the reservoir. The pulse rate accelerated to 115 beats per minute from a normal of 92 when the low level of oxygen was reached. The rate retarded gradually to 92 during the following 80 minutes. We shall



TABLE 3

*Experiments in the low pressure chamber. Subjects taken to 18,000 feet (380 mm.) in 18 minutes and maintained at that level*

## CIRCULATORY RESPONSES TO LOW OXYGEN TENSIONS

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| NAMES      | DATE     | PULSE |    |    |     |     |     |     |     |     |     | SYSTOLIC |     |     |     |     |     |     |     |     |    | DIASTOLIC |    |    |    |    |    |    |  |  |  |
|------------|----------|-------|----|----|-----|-----|-----|-----|-----|-----|-----|----------|-----|-----|-----|-----|-----|-----|-----|-----|----|-----------|----|----|----|----|----|----|--|--|--|
|            |          | 0     | 5  | 10 | 15  | 25  | 35  | 55  | 75  | 95  | 0   | 5        | 10  | 15  | 25  | 35  | 55  | 75  | 95  | 0   | 5  | 10        | 15 | 25 | 35 | 55 | 75 | 95 |  |  |  |
| N. E. F.   | 6/7/18   | 73    | 75 | 75 | 79  | 82  | 79  | 76  | 72  |     | 114 | 112      |     |     |     | 100 | 102 | 104 | 100 | 70  | 72 |           |    | 56 | 58 | 58 | 54 |    |  |  |  |
| R. S. S.   | 6/10/18  | 70    | 74 | 76 | 80  | 94  | 90  | 95  | 88  |     | 118 |          | 114 | 114 | 110 | 104 | 100 |     | 70  |     |    | 64        | 54 | 48 | 42 | 42 |    |    |  |  |  |
| M. G. B.   | 6/11/18  | 72    | 74 | 78 | 82  | 90  | 88  | 86  | 82  | 97  | 118 |          |     |     |     | 112 | 112 | 110 | 112 | 112 |    |           |    | 66 | 68 | 62 | 64 |    |  |  |  |
| W. B. M. 1 | 6/12/18  | 62    | 62 | 62 | 68  | 78  | 78  | 78  |     | 118 |     |          |     |     |     | 118 | 118 | 112 | 118 | 82  |    |           |    | 72 | 58 | 56 | 54 |    |  |  |  |
| A. W. L.   | 6/18/18  | 93    | 93 | 96 | 98  | 98  | 100 | 101 | 101 | 118 | 120 | 122      | 124 | 120 | 116 | 116 | 118 |     | 76  |     |    |           | 78 | 64 | 56 | 60 |    |    |  |  |  |
| B. M. L.   | 6/17/18  | 68    | 69 | 70 | 78  | 89  | 90  | 87  | 85  | 102 |     |          |     |     |     | 102 | 102 | 102 | 102 | 68  |    |           |    | 52 | 48 | 46 | 46 |    |  |  |  |
| G. C. W.   | 6/25/18  | 60    | 62 | 64 | 70  | 78  | 78  | 82  | 84  | 100 |     |          |     |     |     | 120 | 120 | 118 | 114 | 60  |    |           |    | 64 | 58 | 48 | 48 |    |  |  |  |
| E. W. B.   | 6/30/18  | 75    | 80 | 81 | 84  | 88  | 86  | 82  | 84  | 120 |     |          |     |     |     | 144 | 134 | 130 | 150 | 74  |    |           |    | 74 | 62 | 64 | 78 | 70 |  |  |  |
| J. M.      | 7/1/18   | 75    | 78 | 87 | 90  | 98  | 86  | 86  | 82  |     | 130 | 126      | 124 | 124 | 136 | 130 |     |     | 70  |     |    |           | 60 | 52 |    |    |    |    |  |  |  |
| B. F.      | 7/2/18   | 78    | 78 | 84 | 100 | 123 | 114 | 106 |     | 114 | 112 | 112      | 114 | 118 | 116 | 116 | 114 |     | 80  |     |    |           |    |    |    |    |    | 78 |  |  |  |
| D. T. R.   | 7/3/18   | 69    | 72 | 72 | 76  | 87  | 87  | 90  | 88  | 108 |     |          |     |     |     | 112 | 114 |     | 64  |     |    |           |    |    |    |    |    |    |  |  |  |
| H. J. M.   | 7/5/18   | 80    | 83 | 89 | 93  | 100 | 97  | 92  |     | 104 |     |          |     |     |     |     |     |     | 70  |     |    |           |    |    |    |    |    |    |  |  |  |
| A. F. H.   | 7/8/18   | 72    | 72 | 75 | 87  | 88  | 87  | 84  | 84  | 110 |     |          |     |     |     | 118 | 118 | 112 | 112 | 90  |    |           |    | 64 | 68 | 70 | 78 | 64 |  |  |  |
| P. S. B.   | 7/9/18   | 75    | 78 | 79 | 81  | 89  | 89  | 87  | 82  | 110 |     |          |     |     |     |     |     |     | 70  |     |    |           |    |    |    |    |    |    |  |  |  |
| W. H. G.   | 7/10/18  | 78    | 82 | 90 | 97  | 100 | 98  | 96  | 93  | 94  | 110 | 110      | 108 | 114 | 120 | 116 | 116 | 114 | 110 | 70  |    |           |    | 64 | 68 | 70 | 61 | 64 |  |  |  |
| E. A. R.   | 7/13/18  | 72    | 72 | 70 | 88  | 90  | 92  | 88  | 86  | 120 | 120 | 118      | 118 | 124 | 118 | 116 | 116 |     | 72  |     |    |           |    |    |    |    |    |    |  |  |  |
| A. F. H. 2 | 7/15/18  | 69    | 69 | 72 | 72  | 78  | 81  | 79  | 72  | 106 | 100 | 98       | 98  | 100 | 104 | 102 | 108 |     | 70  |     |    |           |    |    |    |    |    |    |  |  |  |
| W. C. W.   | 7/16/18  | 66    | 69 | 72 | 75  | 88  | 86  | 82  | 90  | 104 |     |          |     |     |     | 106 | 104 | 102 | 104 | 70  |    |           |    |    |    |    |    |    |  |  |  |
| L. G. R.   | 7/19/18  | 90    | 93 | 93 | 93  | 96  | 92  | 95  | 92  | 114 |     |          |     |     |     |     |     |     | 70  |     |    |           |    |    |    |    |    |    |  |  |  |
| L. J. S.   | 7/26/18  | 65    | 70 | 73 | 78  | 95  | 95  | 93  |     | 110 |     |          |     |     |     | 100 | 102 | 100 | 100 | 82  |    |           |    | 78 | 78 | 76 | 74 |    |  |  |  |
| A. F. H. 3 | 7/30/18  | 78    | 81 | 86 | 86  | 87  | 90  |     |     | 112 |     |          |     |     |     | 112 | 108 | 108 | 96  | 68  |    |           |    |    |    |    |    |    |  |  |  |
| W. B. M. 2 | 7/31/18  | 60    | 62 | 62 | 68  | 82  | 76  | 80  |     | 126 |     |          |     |     |     | 114 | 114 |     | 68  |     |    |           |    |    |    |    |    |    |  |  |  |
| B. R. L.   | 8/5/18   | 84    | 85 | 88 | 90  | 86  | 84  |     |     | 83  | 104 | 102      | 100 | 102 |     |     |     |     | 62  |     |    |           |    |    |    |    |    |    |  |  |  |
| W. B. M. 3 | 12/27/18 | 69    | 69 | 78 | 90  | 110 | 100 | 100 | 96  |     |     |          |     |     |     |     |     |     | 62  |     |    |           |    |    |    |    |    |    |  |  |  |
| E. C. S.   | 4/29/18  | 76    | 76 | 80 | 84  | 98  | 100 | 108 | 105 | 108 |     |          |     |     |     | 128 | 134 |     | 126 | 62  |    |           |    |    |    |    |    |    |  |  |  |
| K. O. N.   | 4/30/18  | 84    | 84 | 89 | 94  | 102 | 98  | 95  | 92  | 116 |     |          |     |     |     | 122 | 120 | 116 | 116 | 68  |    |           |    |    |    |    |    |    |  |  |  |
| N. E. B.   | 5/6/19   | 74    | 74 | 76 | 79  | 86  | 85  | 85  | 84  | 108 |     |          |     |     |     | 112 | 106 | 104 |     | 76  |    |           |    |    |    |    |    |    |  |  |  |



attempt to account for this falling off in rate during the holding period in a later paper.

The striking similarity of the pulse rate responses to a change in barometric pressure and to a decrease in oxygen percentage under normal atmospheric pressure is shown in our experiments with a gradual decrease in oxygen tension, and in the experiments in which a constant low oxygen tension was maintained. The changes in the partial pressure of oxygen give the only adequate explanation. Decreased barometric pressure and lowered oxygen percentage at 760 mm. Hg. result in the same effect on the partial pressure of oxygen, both that of the respired air and also of the alveolar air. Therefore no difference in bodily response to the two methods of producing low oxygen tension should be expected. The low pressure chamber and rebreathing experiments show definitely that barometric pressure *per se* is not a factor.

It has been observed in a number of our experiments in the low pressure chamber that, when the pulse rate falls very definitely, the color of the subject improves. Improvement of cyanosis is suggestive of better oxygenation of the blood.

*Changes in arterial pressures during a gradual reduction of the oxygen tension.* The blood pressures during exposure to the low oxygen of rebreathing have been described by Schneider (12). He reports three types of circulatory reaction to this form of oxygen want.

The first, the optimum, in which the pulse rate accelerates moderately as the oxygen decreases, the systolic pressure is unchanged or shows a terminal rise of not more than from 20 to 30 mm. Hg., and the diastolic pressure remains unchanged or rises slightly. The second, the controlled diastolic fall, in which the pulse rate accelerates moderately and the systolic pressure rises as the diastolic pressure gradually falls. The third, the fainting type, in which after a period of fair, good or excessive response in the rate of heart beat to low oxygen the diastolic pressure suddenly falls and soon thereafter the systolic pressure, and the pulse rate slows.

The optimum type was found to be able to tolerate as low oxygen as 6 per cent, and to lose consciousness without fainting. The fainting type rarely endured as low an oxygen percentage. The pulse pressure was found to remain fairly constant until the oxygen had fallen to between 12 and 9 per cent, after which it increased in amount during the further reduction of oxygen.

In our comparative experiments, by means of low barometric pressures in the low pressure chamber and with low oxygen percentage by the rebreathing and Dreyer methods in which the decrease was grad-

ually carried on until the subject became inefficient, we obtained by each method examples of the types of circulatory reaction described above. The parallelism in the arterial pressures is shown in tables 1 and 2 and figure 1. The parallelism in arterial pressures also emphasizes the fact that barometric pressure in itself is not the causative factor.

*Changes in arterial pressure during an exposure to a constant low oxygen tension.* Corbett and Bazett (13) studied aviators by means of the Dreyer method and found during a period of low oxygen tension, as a general rule, "the pulse pressure increased by a lowering of the diastolic pressure." The systolic pressure showed a slight rise. In cases in which the pulse pressure rise was due to an increase in the systolic pressure, they attributed it to mental or physical work. They believe that the

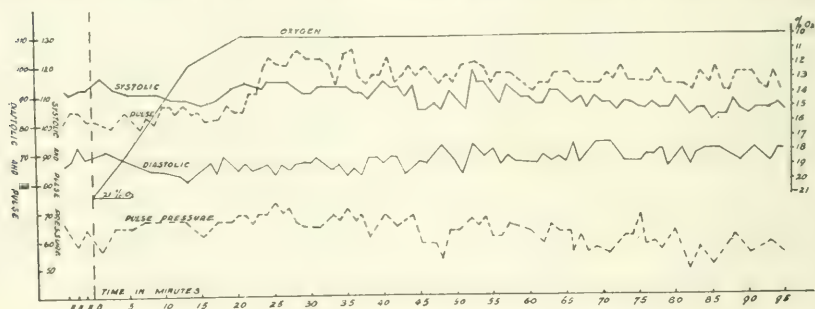


Fig. 3. W. A. B. Taken by the nitrogen dilution method to 10 per cent oxygen (19,400 feet) in 22 minutes and maintained at that level. The pulse pressure gradually decreased after the low oxygen level was reached due to convergence of the blood pressures. The diastolic pressure was little changed during the holding period. See table 5.

diastolic pressure never falls below a level of 60 mm. Hg. in a good type of reaction.

We have tabulated our arterial pressure data for the low pressure chamber and other low oxygen experiments, in which a constant level was maintained, in tables 3, 4 and 5. Since the arterial pressure variations seem to be the same for the several methods of experimentation, they may be discussed collectively.

The systolic pressures run certain clearly defined courses which may be grouped in three classes. The majority of men maintained their normal systolic pressure throughout the entire exposure to low oxygen. A few of these showed a fall at the end of the test and this was associated with oncoming syncope. In approximately 25 per cent of all



cases there occurred a slight rise in the systolic pressure during the middle of the holding period, and then during the last part of the experiment a gradual but slight fall in the pressure took place. A third group showed a gradual fall in the systolic pressure after the low oxygen level was reached. In some of these cases the fall, although slight, from 118 to 100 mm. in one, was progressive throughout the test. In others the fall appeared during the mid-period, after which the pressure held on a level until the close of the experiment.

The variety of systolic pressure changes during short exposure to low oxygen calls to mind the early observations on systolic blood pressure made at high altitudes. There was first a lack of agreement; some found an increase, others no change, and still others a fall in the systolic pressure during the sojourn at a high altitude. Schneider and Sisco (8) working on Pike's Peak concluded that in the majority of healthy men the arterial pressures are unchanged at high altitudes but that some men experience a moderate decrease in the systolic and pulse pressures, the diastolic pressure remaining most constant. The variety of systolic pressure changes is to be expected when one considers that the systolic pressure is to a large extent the resultant of other circulatory factors and compensations. The types of circulatory response are undoubtedly influenced by the severity of the low oxygen exposure with respect to the individual. Just as a man may show a different type of response at 22,000 feet from that at 15,000 feet so may two individuals with unequal compensatory ability show different types of response at a given altitude. Some men tolerate 18,000 or 20,000 feet for half an hour with little disturbance except increased heart rate. Others may have a circulatory collapse before 20,000 feet is reached.

The diastolic pressure changes observed during exposure to a constant low oxygen tension also show some variety. It should be borne in mind that during the period when the reduction in oxygen is being made, the diastolic pressure usually is not affected unless the oxygen is very much reduced, 9.5 per cent or less (21,000 feet). In a certain proportion of cases, however, at the tensions of oxygen corresponding to an altitude of 15,000 and 16,000 feet a slight lowering of this pressure may occur.

In our low pressure experiments we have only occasionally had a subject that showed a decrease in diastolic pressure during the period in which the barometric pressure was being lowered to 380 mm. In three out of some twenty cases in which, under normal atmospheric pressure, the oxygen was lowered to 12 and 10 per cent, a slight fall in pressure occurred during the ascent.

During the period of maintained level the diastolic pressure may remain constant, or it may fall slowly to a new level and hold there. Frequently after a subnormal level of varying duration it regains in some degree the normal level. The return may be only slight but it is sometimes complete. In some cases the diastolic pressure falls very slowly throughout the entire experiment, and in a few instances the experiment was terminated because of a very sudden and decided drop in the pressure.

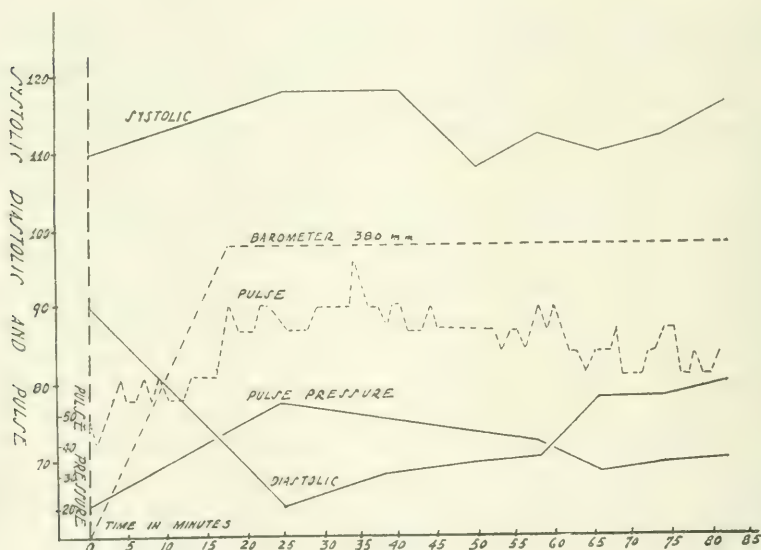


Fig. 4. P. S. B. Taken to 380 mm. (18000 feet) in 18 minutes in the low pressure chamber and maintained at that level. This case illustrates the return of the circulatory factors toward their original values. Compare with K. D. by the rebreathing method, figure 2. See table 3.

Scarcely 20 per cent of all our cases have maintained a constant diastolic pressure throughout the entire experiment. At least 70 per cent of all cases showed a fall in the diastolic pressure during the holding period. The time of its onset was variable. As pointed out, it sometimes began during the ascent, it frequently began during the first five minutes of the hold and in some instances the fall did not begin for 25 or 30 minutes. The fall in diastolic pressure takes place gradually. It requires, as a rule, 10 or more minutes to reach the level. The amount of fall in the diastolic pressure in our low barometric pres-

sure experiments ranged from 4 to 28 mm. Hg. It averaged 12 mm. at 380 mm. In the experiments with low oxygen at normal atmospheric pressure the fall in diastolic pressure varied between 6 and 26 mm. Hg.

The partial or occasional complete return of the diastolic pressure toward the normal after a period of lowered pressure was observed in at least a third of the low pressure experiments and in over 50 per cent of the experiments in which the oxygen percentage had been lowered. We believe that the return of diastolic pressure toward its normal level occurred in those cases which compensated best to low oxygen. The gradual continuous and the sudden terminal fall in diastolic pressure are associated with poor compensation.

The pulse pressure, during the holding period in both kinds of experiments, showed three rather frequent conditions which were chiefly dependent upon the course of the diastolic pressure. When the diastolic pressure was constant, the pulse pressure was, as a rule, constant. In the cases in which the diastolic pressure fell, there was a corresponding rise in the pulse pressure and when it tended to return to normal after a subnormal period, the pulse pressure fell proportionately. The decrease after the preliminary rise occurred in the majority of cases. In exceptional cases the pulse pressure either held its increase throughout or continued to increase slightly until the close of the experiment. There were five cases in which for a time the pulse pressure fell below the normal (see B. R. L. and A. F. H., table 3; V. D., table 4; W. A. B. and C. W. A., table 5). In each of these the subnormal pulse pressure period was the result of a fall in the systolic pressure.

Fainting occurred four times during the holding period at 380 mm. Hg. In these the pulse rate suddenly decreased and the character of the pulse changed. The systolic and diastolic pressures fell far below normal, the diastolic fall preceding, as a rule, the systolic. Administration of oxygen quickly restored the subject.

The above observations on the heart rate and the arterial pressures indicate that a reduction in available oxygen calls forth definite circulatory responses. The work of the circulatory system is governed by the needs of the tissues. The demand for oxygen in sufficient amounts to maintain metabolic activity remains constant during rest. When the supply of available oxygen is suddenly reduced, the tissue oxygen demand must be met by compensatory responses which will supply these needs. This burden of meeting the demand for oxygen could be taken over within limits by the circulatory mechanism. If

TABLE 5  
*Experiments by the diluted nitrogen method. Subjects taken to 10 per cent oxygen in 17 to 24 minutes and maintained at that level*

| NAME     | DATE    | PULSE |    |    |     |     |     |     |     |    |     | SYSTOLIC |     |     |     |     |     |     |     |    |    | DIASTOLIC |    |    |    |    |    |    |  |  |  |
|----------|---------|-------|----|----|-----|-----|-----|-----|-----|----|-----|----------|-----|-----|-----|-----|-----|-----|-----|----|----|-----------|----|----|----|----|----|----|--|--|--|
|          |         | 0     | 5  | 10 | 15  | 25  | 35  | 55  | 75  | 95 | 0   | 5        | 10  | 15  | 25  | 35  | 55  | 75  | 95  | 0  | 5  | 10        | 15 | 25 | 35 | 55 | 75 | 95 |  |  |  |
| W. A. B. | 5/31/18 | 80    | 81 | 84 | 84  | 102 | 99  | 96  | 93  | 93 | 112 | 110      | 110 | 108 | 114 | 112 | 110 | 104 | 104 | 68 | 66 | 61        | 62 | 64 | 61 | 68 | 68 | 68 |  |  |  |
| I. M. M. | 5/27/18 | 90    | 93 | 99 | 100 | 105 | 116 |     |     |    | 130 | 126      | 124 | 128 | 132 | 110 |     |     |     | 88 | 88 | 82        | 80 | 80 | 68 |    |    |    |  |  |  |
| A. E. C. | 6/1/18  | 79    | 79 | 79 | 79  | 90  | 94  | 94  | 92  | 94 | 110 | 110      | 106 | 110 | 118 | 112 | 112 | 110 | 106 | 72 | 72 | 72        | 72 | 80 | 72 | 62 | 66 | 64 |  |  |  |
| C. N.    | 5/29/18 | 58    | 62 | 66 | 76  | 84  | 88  | 84  | 84  | 84 | 118 | 118      | 114 | 118 | 114 | 110 | 116 | 118 | 118 | 76 | 72 | 74        | 70 | 66 | 66 | 54 | 62 | 50 |  |  |  |
| G. B. H. | 5/28/18 | 88    | 90 | 92 | 95  | 103 | 102 | 98  | 102 |    | 110 | 112      | 112 | 110 | 112 | 116 | 110 | 104 |     | 70 | 70 | 70        | 66 | 66 | 64 | 62 | 54 |    |  |  |  |
| E. A. R. | 6/4/18  | 68    | 70 | 72 | 73  | 74  | 78  | 78  | 78  |    | 106 | 112      | 110 | 110 | 104 | 104 | 110 | 112 |     | 68 | 68 | 70        | 70 | 66 | 62 | 64 |    |    |  |  |  |
| C. W. A. | 5/25/18 | 90    | 90 | 96 | 99  | 104 | 101 | 101 |     |    | 116 | 112      | 108 | 108 | 118 | 118 | 110 |     |     | 84 | 84 | 86        | 82 | 82 | 78 | 78 |    |    |  |  |  |
| R. S. S. | 5/27/18 | 66    | 66 | 70 | 70  | 86  | 95  | 94  | 88  |    | 124 | 126      | 122 | 118 | 108 | 112 | 112 | 118 |     | 72 | 72 | 72        | 70 | 62 | 56 | 52 | 64 |    |  |  |  |
| I. M.    | 5/28/18 | 72    | 74 | 82 | 84  | 88  | 92  | 103 | 94  | 90 | 112 | 108      | 110 | 110 | 110 | 110 | 114 | 110 | 112 | 80 | 80 | 80        | 76 | 70 | 72 | 72 | 72 | 76 |  |  |  |
| K. D. *  | 1/2/18  | 92    | 92 | 99 | 114 | 108 | 104 | 98  | 92  |    | 142 | 142      | 144 | 152 | 164 | 148 | 142 | 134 |     | 72 | 74 | 74        | 68 | 48 | 50 | 56 | 70 |    |  |  |  |

\* Rebreather to 12.5 per cent oxygen in 18 minutes and held.



the blood contains less oxygen per unit than normally, more blood must flow to the tissues to bring the required amount.

Our experiments give evidence of increased blood-flow in the acceleration of the rate of the heart beat, and in the fall of diastolic pressure with the resulting increase in pulse pressure, which occur in the majority of men held at the low oxygen level. The fall in diastolic pressure is evidence of a peripheral relaxation of the arterioles to allow more blood to pass through the tissues. The increase in the rate of heart beat with such blood pressure relationships as we find in the majority of our examinations is evidence of increased per minute output of the heart.

If this interpretation is accepted, certain features in our experiments will be found suggestive. In a considerable proportion of all men examined the pulse rate, after reaching its maximum, maintained that rate for a time and then slowly retarded. In many of these cases the diastolic fall began either before the heart reached its maximum rate, or when it arrived there. Then followed a period during which the pulse pressure continued to increase while the heart maintained its high rate. It soon took a constant level which was maintained for a period of considerable length. Still later the heart rate slowly retarded and the pulse pressure gradually fell. This would indicate that a marked and progressive increase in the rate of blood flow occurred during the reduction and early holding periods, which was followed by a period of more or less constant rate of flow. Later, as evidenced by the heart retardation and rise in diastolic pressure, the flow of blood returned somewhat toward the normal rate. The validity of this interpretation may be questioned because a retardation in the blood flow occurs when the needs of the tissues for oxygen remain unaltered. The observations on cyanosis should here be borne in mind. At 380 mm. Hg. pressure, some subjects were for a time very cyanotic and later, when the heart rate was retarding and the diastolic pressure rising, the color improved. Some subjects felt wretched during the first part of the holding period only to report later that they were feeling much better. We believe that the retardation of the heart rate, the return of diastolic pressure toward normal, the decrease in pulse pressure and the improvement in color and feeling occur when other compensatory mechanisms have become effective. We plan to report observations concerning the problem in another paper.

## SUMMARY

1. Low oxygen tension effects were studied during a period of gradual decrease and while a level was maintained for from 30 to 130 minutes. Three methods were employed to obtain low oxygen tensions, the low pressure chamber, rebreathing of air at normal atmospheric pressure, and air diluted with nitrogen.

2. The effects of low oxygen tensions upon the circulatory mechanism are the same regardless of the method used to vary the oxygen. Barometric pressure *per se* is not the causative factor.

3. The heart rate responds to slight changes in oxygen tension. The acceleration in the majority of men examined began between oxygen partial pressures of 113 and 128 mm. corresponding to barometric pressures of 542 and 610 mm. (5800 and 8800 feet). In at least 25 per cent of all cases the first response occurred at oxygen partial pressures of about 137 mm. or less corresponding to a barometric pressure of 656 mm. (4000 feet). The initial response occurred at about the same oxygen tension each time a subject was exposed to a decreasing oxygen tension by the several methods used.

4. The increase in rate of heart beat differed in individuals, but was found to be the same for an individual when the rates of decreasing tension and the partial pressures reached were the same in the comparative experiments.

5. When a constant level of oxygen was maintained, the heart reached a maximum rate after the lapse of a period of variable length. This maximum was maintained for some time in the majority of men, after which the rate returned in greater or less degree toward the normal rate. In others the maximum rate once attained was continued to the close of the experiment. A few men showed a gradual increase in rate throughout the whole period of the constant low oxygen level.

6. The systolic pressure maintained its normal level in the majority of cases. In 25 per cent of the cases a slight rise occurred during the first part of the experiment, falling gradually as the experiment proceeded. In others a gradual fall began soon after the desired altitude was attained.

7. The diastolic pressure usually fell gradually about 4 to 28 mm. during a period of a maintained oxygen tension. In many of the experiments it later returned somewhat toward the normal. In some cases the diastolic pressure continued to fall gradually throughout the entire experiment. In a few men the pressure was unaltered by the low oxygen.

8. The pulse pressure usually increased during the time of the holding period at a given oxygen tension. It ordinarily followed the diastolic pressure changes in an inverse way.

9. The bearing of the pulse rate and blood pressure changes on blood flow are briefly discussed.

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## XVIII. CONDUCTION IN THE SMALL INTESTINE

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During the last year we have brought forward considerable evidence in favor of the view that there is a metabolic gradient in the intestinal wall from duodenum to ileum (1). Per unit of weight and time the duodenal muscle gives off more  $\text{CO}_2$  than does the ileal muscle. That this is not due simply to its greater activity is shown by the fact that the difference can still be shown when both segments are kept paralyzed by adrenalin. It is our belief that this metabolic gradient underlies and gives rise to gradients of rhythmicity, latent period and irritability which determine the direction of the diastaltic waves.

It next occurred to us that such a metabolic gradient might influence the conduction of stimuli up and down the intestine. We should expect such stimuli to travel farther and faster with the gradient than against it. It seemed worth while to look into the matter not only because of its academic interest but because the demonstration of such differences in conduction might throw light upon the mechanism of the "myenteric reflex" and upon various clinical problems. When we contemplate the tremendous advances which have been made in medical knowledge through the study of conduction in the heart muscle we must the more lament our almost complete ignorance as regards conduction in the intestine.

Schillbach (2) thought the contractions resulting from electrical stimulation of the bowel ran farther upwards than downwards. Bayliss and Starling (3) found it hard to show descending inhibition in the rabbit because it extended at most 2 to 3 cm. and was so fleeting that it did not alter the appearance of the tracing to any great extent. In no case in the rabbit did excitation spread more than 5 to 6 cm. upwards. They had difficulty in showing any spread of the stimulus in the cat's bowel unless they gave castor oil. In the dog, the descending impulses traveled farther than the ascending. In fact, they could



not show the ascending excitation unless they stimulated just aborally to the recording balloon. On the other hand, they sometimes obtained effects two or three feet below the point stimulated (4). Conduction was slow—about 10 cm. per second. They had difficulty in estimating this rate on account of the long and variable latent period. After painting the bowel with cocain or after injecting the animal with nicotine the waves seemed to run equally well in either direction at a rate of 2.3 cm. per second. Stimuli evoked little response in these poisoned bowels.

There are a number of observations in other fields which would lead us to expect a better conduction in the aboral than in the oral direction. Child (5) has shown in many tissues that there is a constant stream of inhibiting influences descending along the gradients of metabolism which he has demonstrated. Tashiro (6) has explained the polarity of nerves on the same basis. The gradient of  $\text{CO}_2$  production descends peripherally in motor nerves and descends centrally in sensory nerves. MacArthur and Jones (7) have shown a gradient of oxygen consumption in the central nervous system descending from the brain to the end of the cord. When angle worms (8), planarians and centipedes (9) are cut in two, the forward end may crawl on undisturbed while the hind end writhes convulsively. One explanation of this phenomenon is that conduction is almost entirely in the aboral direction. Carlson (9) has shown in the myriapoda that conduction along the ventral nerve cord is more rapid in the aboral than in the oral direction. This is true also for the spinal cord of the hag-fish (10) and probably for the cord of the snake (11). Of course in the spinal cord this difference might perhaps be due to a greater length and simplicity of the downward conducting paths. Sherrington has shown with the spinal cord of higher animals that inhibition spreads downward more easily than upward. In the decerebrate cat it is much easier to obtain reflexes in the hind limbs by touching the head than to get movements of the head or fore-limbs after touching the tail. "The exclusively aboral direction taken by shock seems to be universal in the nervous system" (12). It is suggestive that in the cord the region just above an injury seems often to be in a stimulated and hyperesthetic state much as the corresponding segment should be in the bowel.

The simplest nerve nets such as those in the medusae and sea-urchins conduct stimuli equally well in all directions (13). Others, however, like those in the feet of snails are "polarized" so that they conduct best in one direction (14). Parker (15) has suggested that the direc-

tion of peristalsis may be due to such a polarization of Auerbach's plexus.

*Technic.* Most of the work has been done on the small intestine of the rabbit because its contractions are so even and regular that abrupt changes in the record can with considerable certainty be interpreted as the effects of the stimuli used. Many experiments have been done also with the bowel of the cat. The rat's intestine was so insensitive to stimuli that we made only a few attempts to use it.

In order to simplify the problem we first studied the conduction in excised loops of bowel contracting rhythmically in warm aerated Locke's solution. Such a loop is fastened to the bottom of the vessel by serrefines which grasp it at two points 3 cm. distant from the ends. These short end segments are then turned up like the vertical arms of a U and connected to heart levers by means of threads. By turning up the recording ends of the loop in this way, one can avoid the use of pulleys.

In order to get a longer stretch of bowel between the recording ends and to have the bowel more accessible for the various types of stimulation, we replaced the usual tall narrow beaker with a long, comparatively shallow, glass bread-baking dish. On the bottom of this dish was a strip of cigar-box wood held down with lead weights. A centimeter scale was marked on its upper surface and at one end of the scale was fastened a little wire serrefine. At the other end was nailed a wooden upright with a little ring at its base and a cleat at the top. The segments were fastened in this apparatus as shown in figure 1. The water-bath surrounding the glass dish was kept at 38°C.

In some experiments the bowel was stimulated by pinching or by applying a crystal of NaCl. The most convenient form of stimulation and the only measurable one was the faradic shock. A convenient electrode for underwater work was made by inserting the wires from the secondary coil into two L tubes the lower ends of which were fastened on a short bar so that the bowel would just fill the space left between them. (See fig. 1.) A few experiments showed that the current kept very closely to the short path between the two tubes. A Harvard induction coil was used with one dry cell supplying 20 amperes at 1.7 volts. A tetanizing current was used.

In most of the tracings the movements recorded are those of the longitudinal muscle. When the serrefines were placed so that the circular muscle was recording, the amplitude of contraction naturally was less; the tendency to beat rhythmically was less and the irritability

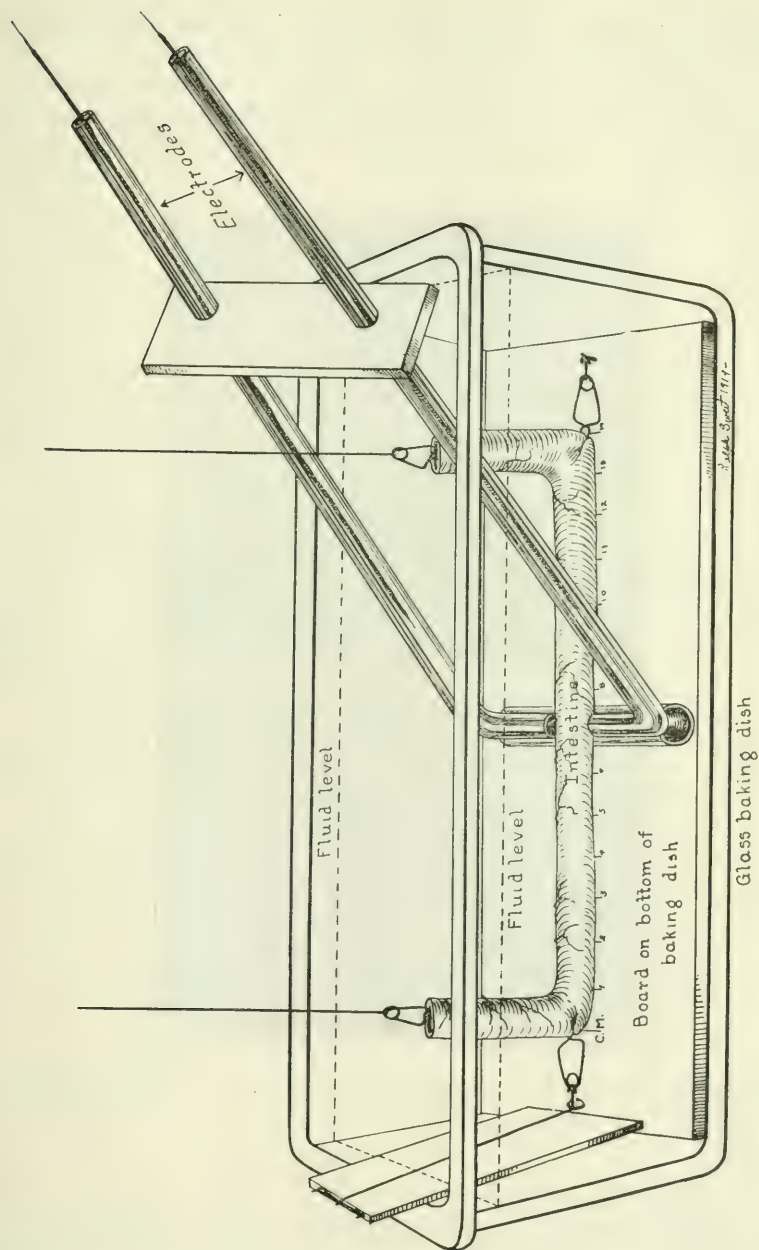


Fig. 1. Apparatus used in studying excised segments. For details see the text.

was often very low. Otherwise the results of stimulation were about the same as those obtained with the longitudinal muscle.

The work on the excised segments was repeated later on the intact bowel. The animals were anesthetized with urethane (2 gm. per kilo by mouth) and the cord was destroyed below the 4th dorsal vertebra.

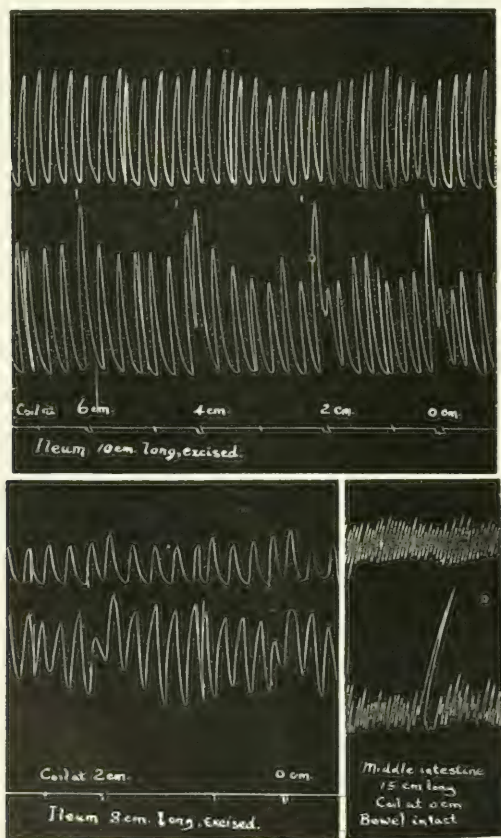


Fig. 2. When the bowel is stimulated midway between the recording segments the effects are most marked on the aboral end. The upper tracing is from the orad end.

The abdomen was opened under a bath of Locke's solution kept at 38°C. The best records were obtained with a very simple apparatus. Two glass rods were held vertically with their lower ends in the animal's abdomen. Fastened to the ends were little serrefines which seized the bowel at two points from 5 to 50 cm. apart, and held it down. Other



serrefines, attached by threads to the levers, were so arranged that two segments, 3 cm. long, and the desired distance apart, recorded their contractions on the drum. In some experiments we used the enterograph designed by Alvarez (16); in others we used balloons.

*Conduction better in the aboral direction.* The conclusions reached in this paper are based upon an analysis of over 2200 reactions. There were about 1000 each on the excised and intact intestines; 1700 were the results of electric stimuli; 400 the results of pinches and cuts, and the rest were the results of stimulation by salt crystals and balloons. In practically every instance it was easy to show that conduction is better in the caudal direction. If the distance between recorders was small enough so that a stimulus in the middle affected both tracings, the disturbance was more marked in the lower one than in the upper. (See fig. 2.) With a longer distance between the two, the lower recording segment would respond well to stimulation at the base of the upper when the upper failed to respond to stimulation at the base of the lower. (See fig. 3.) In other experiments the stimulating electrodes were brought closer and closer to the recording segment until it showed some response. The following table shows some of the results obtained in this way with strong faradic stimuli. It will be noticed that in one case a response was obtained 57 cm. below the point stimulated and only 7 cm. above.

| DUODENUM         |        | JEJUNUM |        | MIDDLE |        | ILEUM |        |
|------------------|--------|---------|--------|--------|--------|-------|--------|
| Orad             | Caudad | Orad    | Caudad | Orad   | Caudad | Orad  | Caudad |
| Excised segments |        |         |        |        |        |       |        |
| 5.5              | 12.5   | 5.5     | 15.0   |        |        |       |        |
| 6.0              | 12.5   | 8.0     | 20.0   | 6.0    | 12.5   | 6.0   | 12.5   |
|                  |        | 6.0     | 15.0   | 6.0    | 15.0   | 6.0   | 13.0   |
| 10.0             | 18.0   | 6.0     | 15.0   | 6.0    | 15.0   |       |        |
| 7.0              | 12.+   | 8.0     | 12.+   | 7.0    | 12.+   |       |        |
| Intact animal    |        |         |        |        |        |       |        |
| 2.5              | 19.5   |         |        | 2.5    | 14.5   | 2.5   | 10.0   |
|                  |        | 9.5     | 19.5   | 9.5    | 48.0   | 7.0   | 14.5   |
|                  |        | 5.0     | 19.0   | 5.0    | 19.0   | 5.0   | 20.0   |
|                  |        |         |        | 4.5    | 36.0   | 4.5   | 36.0   |
|                  |        | 5.0     | 20.0   |        |        | 5.0   | 24.0   |
|                  |        |         |        | 5.0    | 43.0   |       |        |
|                  |        | 9.5     | 43.0   | 10.0   | 43.0   | 12.0  | 72.0   |
|                  |        |         |        | 7.0    | 57.0   |       |        |

The figures on any horizontal line are from one rabbit.

These differences were observed also with the mechanical and chemical stimuli, but they were not so striking. The experiments in which only the hind end of a worm reacts to a cut can sometimes be duplicated with a short U-loop of excised bowel.

*The myenteric reflex.* It will be noticed in the tracings that the characteristic response not only at the point stimulated but in the regions proximal and distal to it is an increase in amplitude and tone followed perhaps by a drop in amplitude and tone. In all this work we have seen very few examples of what might be called a "myenteric reflex." Thinking that perhaps this was due to the fact that we were using the longitudinal muscle when previous workers had used the

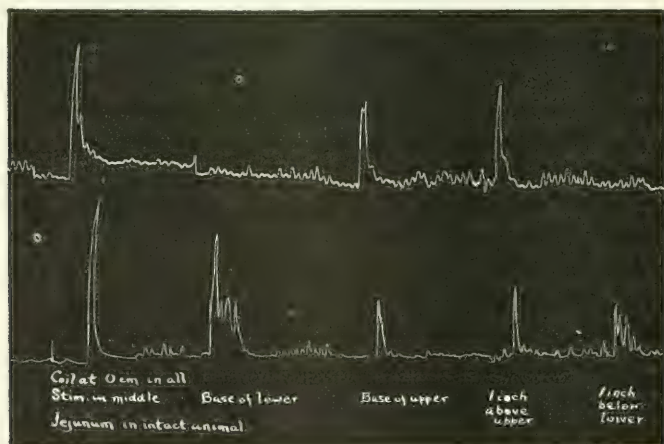


Fig. 3. When the bowel is stimulated above it responds below; when stimulated below it often fails to respond above. The upper tracing is from the oral segment. The distance between the recording segments was 5 cm.

circular we repeated the work attaching the serrefines so they would pull at right angles to the long axis of the bowel. In some experiments also we used balloons as others have done. With the excised segments the circular coat was slow to start beating and was often very unresponsive to stimuli. Otherwise the results were usually the same as those obtained with the longitudinal muscle. The greatest number of typical myenteric reflexes were obtained while using balloons—suddenly inflated and deflated—as the stimulus. We were not entirely satisfied, however, with the results obtained with these balloons. The trauma attendant upon their insertion and the violent

efforts of the bowel to force them out make conditions far from normal. It may easily be that the "myenteric reflex" is primarily a response to stretching and not a response to other forms of stimulation of the gut.

*Observations on different animals.* The rabbit's colon was too insensitive for satisfactory work. We had the same trouble with the excised small bowel of dogs and white rats. Considerable work was done with cats. The excised small intestine beat irregularly and was often insensitive. We were able to show, however, the same peculiarities of conduction that were seen in the rabbit. One cat was decerebrated so that we could avoid the use of urethane. As our results with this animal were the same as with others, the anesthetic probably had no appreciable effect on the bowel.

Remarkable differences were found in the irritability and conductivity in different animals of the same species. Previous work makes us feel that the degree of infection with intestinal and other parasites must have a good deal to do with these differences. Often the bowel was very insensitive in spite of a good rhythmicity.

Both with the excised segments and intact animals conduction seemed better in the middle region of the small intestine than at the ends. Poor results in the duodenum and upper jejunum could easily be explained, however, by the greater reaction to trauma and handling in that region. Sometimes the duodenum would respond well only to the first stimulus.

*Rhythmicity.* We had occasion to confirm the previous observation of Alvarez (17) that the rate of the oral end of an excised segment of rabbit's intestine is higher than the rate of the lower end. Keith (18) has suggested, on the basis of his anatomical studies and some of Alvarez's observations, that there are a number of rhythmic centers in the bowel, containing nodal tissue and dominating the rhythm of the adjacent regions. We have not been able to show any such dominance anywhere except occasionally in the terminal ileum (17). When the aboral recording end of a segment was severed from the rest of that segment its rate of contraction was never altered to any extent. There seem to be no such descending influences affecting the rhythm as there are in the heart. It is an interesting point, however, that when the longer segments were put into the aerated Locke's solution the lower end usually started contracting some time before the upper. This was due probably to a greater reaction to trauma caudad to the upper cut end than oral to the lower cut end.

*Rate of conduction.* Theoretically, an impulse should not only travel



farther with the gradient but it should travel faster with it than against it. We have made many attempts to show this but so far have not been able to obtain trustworthy records. The normal bowel is constantly in motion so that no satisfactory base line can be maintained. The muscle is slow in its reactions so that it is hard to say just when it begins to shorten. When it is contracting rhythmically a stimulus may show itself only as an increase in the height of the succeeding wave. Sometimes there is a temporary inhibition which delays the appearance of the reinforced wave. Still stronger inhibition may show itself as a drop in tone before the rise. But even when a fairly sharp take-off can be marked on the tracing the variations in latent period are so large that they may cover up differences due to the conduction time. Moreover, the latent period at a distance from the point stimulated may be lengthened considerably because the stimulus has become weakened during conduction. The different types of response to stimuli are shown in figure 4. A good deal of work was done with segments in which the rhythmic contractions had been more or less stilled by strychnin, adrenalin, nicotin, cocain and digitalis. Segments were studied also in baths of physiologic NaCl solution where they did not beat. Although some fairly satisfactory records were obtained in this way, slight tonus changes generally persisted even when the segments were so badly poisoned that their irritability and conductivity were practically gone. The most conclusive data were obtained from records of actively contracting untreated segments, stimulated half way between the two ends. Ordinarily, on account of the difference in rate at the two ends of the strip, one lever would be going up while the other was coming down, but occasionally for a few seconds the two ends would beat practically in unison. If stimuli were thrown in at those times it could often be shown that a fairly abrupt alteration in the tracing appeared first at the distal end. Following are some of the time intervals obtained. The segments were from 8 to 15 cm. long. The figures represent seconds elapsed after stimulation in the middle.

*Excised segments*

|                |     |     |     |     |
|----------------|-----|-----|-----|-----|
| Upper end..... | 2.0 | 1.5 | 4.2 | 4.2 |
| Lower end..... | 1.2 | 0.5 | 2.7 | 1.5 |

*Intact animal*

|                |      |     |     |
|----------------|------|-----|-----|
| Upper end..... | 0.8  | 1.3 | 1.1 |
| Lower end..... | 0.25 | 1.0 | 0.6 |



The conductivity varied markedly in the different animals. In two of the rabbits the impulse travelled 40 or 50 cm. in less than one second. Ordinarily the conduction time seemed to be much shorter. Following are some of the estimates made: 9, 15, 40, 27, 7, 10, 8, 30, 50, 20, 11,

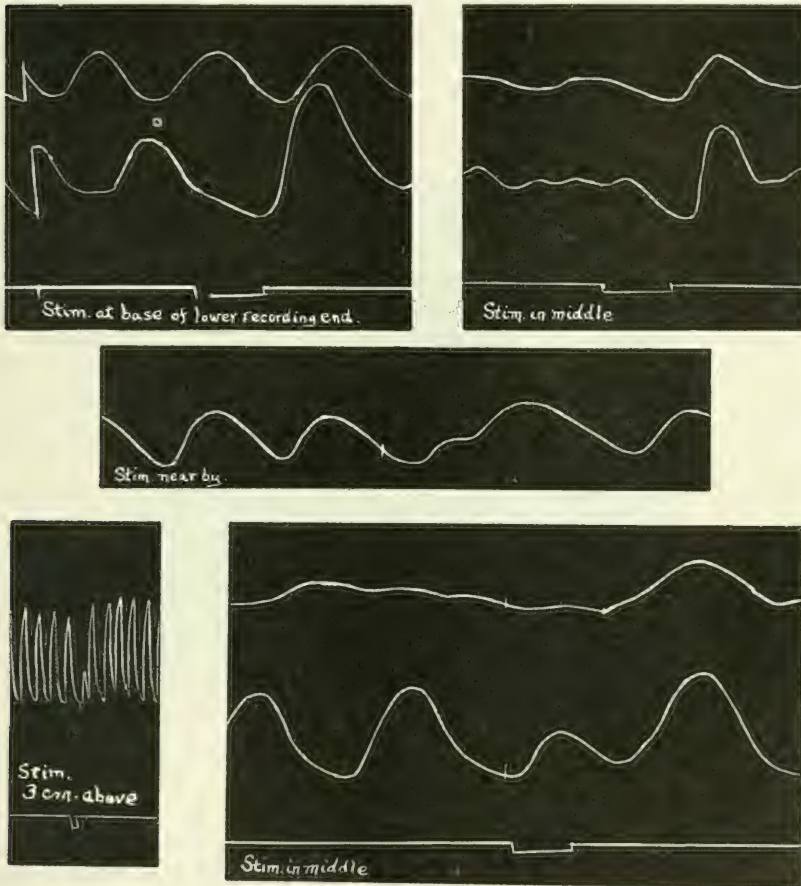


Fig. 4. To show different types of response to stimulation and the difficulties in the way of estimating the conduction time. The upper tracing is from the orad segment.

26, 16, 12, 40, 13, 22, 30, 11, 12, 40, 15. The average is 21 cm. per second. This figure would probably be higher with a more accurate technic. It represents the aboral rate. Conduction orad is harder to

measure as it is ordinarily limited to short distances where the error is larger.

At first sight the slow rate suggests conduction through the muscle, but a review of the literature on nerve-net muscle combinations shows that the impulses generally travel through the net and that the rate is usually slow. Thus the rate in *Cassiopea* is 27 to 50 cm. per second at 25°C.; in *Metridium* at 21°C. it varies between 12 and 14 cm. per second (19). The nerve net not only serves to expedite conduction but it keeps the muscle from contracting down hard. Several observers have noticed that when smooth muscle is cut off from its nervous connections its tone rises to a point where rhythmic contraction is impossible (20). It may be that some of the contractions in spasmodic ileus, in infantile pyloric stenosis and in Hirschprung's disease are due not to some abnormal stimulation but to the loss of this normal inhibition.

Some evidence was obtained at times of a more rapid conduction, perhaps by way of the mesenteric nerves. On two occasions sharp reactions were observed in the lower ileum two seconds after a stimulus had been applied to the greater curvature of the stomach. The distance along the bowel was from 300 to 400 cm. Frequently marked changes in the tone and rhythmicity of the loop studied would follow almost immediately after defecation, after small peristaltic rushes, or after handling the bowel 200 cm. or more above the region observed. The tone of the whole small intestine seemed to rise during efforts at defecation and it fell suddenly when food passed through the ileo-cecal sphincter. Figure 5 shows some of these long distance reactions. Bayliss and Starling have well said that "every point of the intestine is in a state of activity which can be played upon and modified by impulses arriving at it from all portions of the gut above and below" (21).

The rush waves along the bowel in the rabbit travel about 4 cm. per second. Theoretically they should go faster in the duodenum where the gradient is steepest and the metabolic rate fastest. They certainly seem to do so in man where the resultant rapid emptying accounts for the term "jejunum." In the long bowel of the rabbit a rush wave seems often to gain headway the farther it goes so that a wave which travels 2 cm. per second in the duodenum travels 6 cm. per second in the lower ileum.

*No difference in reaction with strychnin.* The work of pharmacologists suggests strongly that strychnin acts mainly upon the synapses between the neurones; it makes conduction across them easier and

therefore heightens reflexes (22). It may be then that we can follow Parker's suggestion (15) and use strychnin as an index to the simplicity or complexity of the nervous system in different animals. The simpler forms of life with pure nerve nets should show only the toxic protoplasmic effects of strychnin, while the higher forms with more and more synapses should show heightened reflexes and clonic spasms. Moreover, we might find that disturbance in the mechanism of reciprocal innervation which has been observed not only in the spinal cord of mammals but even in earthworms and starfish after the administration of strychnin (23). Although considerable work must yet be

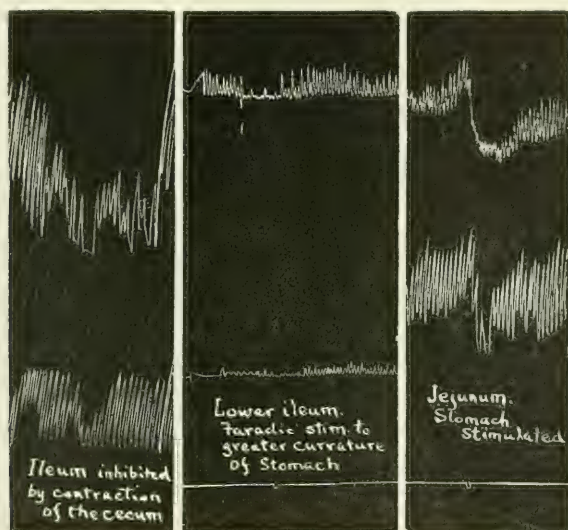


Fig. 5. Intact bowel affected by activity of the cecum and by stimulation of the greater curvature of the stomach.

done before we can say how dependable this strychnin test is, it is certainly suggestive that we have been unable to show much difference between the conduction time and the type of reaction to stimuli in strychninized and normal animals.

We kept a number of our rabbits and cats for several hours under large and repeated doses of strychnin. At no time could we show any improvement in conduction or any change in the type of reaction to stimuli, even when the animals were twitching and going into convulsions. (It should be remembered that they were under urethane



anesthesia.) Similar experiments were performed with the excised segments, beating in Locke's solution to which strychnin had been added. Frequently conduction was decidedly impaired by the drug but almost always it remained better in the caudad than in the orad direction. Auerbach's plexus then would appear to be a simple net, without reflex arcs and without synapses excepting those between the net and the central nervous system. This conclusion agrees with that of the anatomists who, after much argument and research, have finally decided that there are no commissural fibers in the involuntary nervous system such as would be necessary for the working of true reflexes (24).

#### SUMMARY

It has been shown that there is a metabolic gradient in the small intestine from duodenum to ileum. As would be expected, conduction is better with the gradient than against it.

The rate of conduction could not be determined accurately. It appears to be about 20 cm. per second. At times it is about 150 cm. per second, probably by way of the nerves in the mesentery.

In the rabbit, the peristaltic rushes travel about 4 cm. per second.

The characteristic response to a stimulus applied to the gut is a contraction above and below. This may be preceded or followed by an inhibitory phase. The "myenteric reflex" was rarely observed, and then usually after distension by balloons.

The tone and activity of any part of the tract can be affected markedly by the activities of other parts.

With the exception of the terminal ileum, no part of the bowel seems to affect the rate of rhythmic contraction of adjacent parts. This finding is against the theory of peristalsis offered by Keith.

The failure of strychnin to influence conduction or to alter the type of response to stimuli suggests that Auerbach's plexus is a simple nerve-net, without synapses or reflex arcs.

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## RESPIRATORY VOLUMES OF MEN DURING SHORT EX- POSURES TO CONSTANT LOW OXYGEN TENSIONS ATTAINED BY REBREATHING<sup>1</sup>

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Various writers have shown in experiments on men, both with re-breathers and in pneumatic chambers, that an increase in the per minute respiratory volume accompanies rather rapid reduction of the oxygen tension in the air breathed by the subject. Hough ('10, '11) found that the continued rebreathing of a small quantity of air (20 to 30 liters) without the removal of the carbon dioxide produced by the subject, caused a marked increase in the depth of respiration and in the respiratory volume. Twenty-three of his 25 cases showed an increase of respiratory depth from the start of the rebreathing test, and the increase in the depth of respiration was most marked in those cases in which there was some decrease in the rate of respiration. Haldane and Poulton ('08) using a rebreather which removed the carbon dioxide produced by the subject, observed great hyperpnoea when the oxygen in the air breathed was reduced from 10 to 5 per cent in a few minutes. Schneider ('18) reporting on rebreather tests of 25 to 30 minutes duration, states that more than 50 per cent of the men examined gave an increase in respiratory volume when the reduction of oxygen had proceeded to 16 to 14 per cent. Lutz ('19) noted an increase in the respiratory volume of men in the low pressure chamber at a pressure equivalent to 4000 feet altitude, when the pressure was reduced at a rate equivalent to a rise of 1000 feet per minute. Lutz and Schneider ('19)

<sup>1</sup> Abstract, in part, in Proc. American Physiological Society, this Journal, p. 119, vol. xlix, 1919, by authority from S. G. O., dated April 23, 1919.

in a series of experiments both in the low pressure chamber and with the Dreyer nitrogen apparatus found that the onset of increased breathing occurred at about 656 mm. mercury pressure (about 4000 feet altitude) when the reduction in oxygen tension had been made at a rate equivalent to a rise of 1,000 feet per minute. They also state that 9 out of 14 subjects gave a maximum ventilation during the first ten minutes at 20,000 feet, and that following this period there was a distinct falling off in the per minute volume.

The data which follow afford comparisons of the per minute respiratory volumes of 29 men during exposures of 10 to 30 minutes to various constant oxygen tensions lower than the tension of sea level air, with the volumes breathed at sea level and during the reduction of the oxygen tension by rebreathing. Additional data are offered on the increase in respiratory volume during the reduction of oxygen tension by rebreathing. These observations were made as part of the general study of aviation physiology at the Medical Research Laboratory of the Air Service, during the fall and winter of 1918-19. The writer is indebted to Lieutenants H. Fried, C. N. Larsen and B. R. Lutz for assistance during the experimentation.

The rebreather designed by Larsen and Davis (Larsen, '19) was used in these tests. This machine is a portable modification of a closed rebreather in which the carbon dioxide produced by the subject while rebreathing a given volume of air, is removed by a sodium hydroxide cartridge. The rebreather tank contained 54 liters of sea level air at the beginning of each experiment, a volume which the average subject could reduce from 21 per cent oxygen to 9.8 per cent oxygen (equivalent to 20,000 feet altitude) in 20 to 25 minutes. While holding the subject at sea level or at a given oxygen tension, oxygen was supplied automatically to the subject by his own respiratory movements from a balanced oxygen spirometer.

The general plan of each experiment may be summarized as follows. The subject, either sitting or reclining, was connected with the rebreather through a rubber mouthpiece and standard rubber gas-mask tubes. The rebreather spirometer was set at zero and the valves to the subject opened at the end of an inspiration. In this way the spirometer was raised by the volume of one expiration minus the carbon dioxide which was removed as the air passed in through the sodium hydroxide cartridge. One or two inspirations sufficed to lower the rebreather spirometer to zero again, and as the water seal on the oxygen spirometer was broken each time the rebreather spirometer reached the



TABLE 1

*Average per minute respiratory volume in cubic centimeters; subject sitting*

| SUBJECT               | INITIAL 10 MINUTE PERIOD |                     | REBREATHING PERIOD VOLUMES |                     |                    | AT REDUCED O <sub>2</sub> LEVEL |                 |                     | TIME OF REDUCTION |
|-----------------------|--------------------------|---------------------|----------------------------|---------------------|--------------------|---------------------------------|-----------------|---------------------|-------------------|
|                       | O <sub>2</sub>           | Volume              | 1-5 minutes                | 1-10 minutes        | 10th minute to end | Volume                          | O <sub>2</sub>  | Equivalent altitude |                   |
|                       | <i>per cent</i>          |                     |                            |                     |                    |                                 | <i>per cent</i> | <i>feet</i>         | <i>minutes</i>    |
| AX.....               | 21.0                     | 6,100               | 5,840                      | 5,720               |                    | 5,600                           | 17.4            | 5,000               | 9                 |
| AE <sub>1</sub> ..... | 21.0                     | 9,060               | 12,240                     | 14,640              |                    | 13,690                          | 17.4            | 5,000               | 11                |
| C.....                | 21.0                     | 6,140               | 9,200                      | 10,700              |                    | 8,120                           | 15.2            | 8,400               | 11                |
| D.....                | 21.0                     | 6,620               | 9,000                      | 9,460               | 9,500              | 7,700                           | 15.4            | 8,000               | 10                |
| AF <sub>1</sub> ..... | 21.0                     | 7,830               | 7,800                      | 9,530               | 10,900             | 11,100                          | 15.4            | 8,000               | 15                |
| AF <sub>1</sub> ..... |                          |                     |                            |                     |                    | 10,350 <sub>a</sub>             |                 |                     |                   |
| B.....                | 21.0                     | 4,400               | 5,630                      | 7,880               | 7,850              | 7,010                           | 14.8            | 9,000               | 14                |
| AB.....               | 21.0                     | 9,070               | 10,750                     | 12,590              | 12,913             | 12,540                          | 14.3            | 10,000              | 17                |
| AB.....               |                          |                     |                            |                     |                    | 10,006 <sub>b</sub>             |                 |                     |                   |
| AG.....               | 21.0                     | 6,550               | 7,230                      | 8,910               | 8,440              | 8,710                           | 14.3            | 10,000              | 15                |
| AG.....               |                          |                     |                            |                     |                    | 6,750 <sub>b</sub>              |                 |                     |                   |
| AG.....               |                          |                     |                            |                     |                    | 7,360 <sub>c</sub>              |                 |                     |                   |
| AA.....               | 21.0                     | 12,470              | 16,200                     | 16,440              | 17,600             | 16,990                          | 14.3            | 10,000              | 12                |
| AA.....               |                          | 12,720 <sub>d</sub> |                            |                     |                    |                                 |                 |                     |                   |
| AE <sub>2</sub> ..... | 17.4                     | 13,690              | 16,540                     |                     |                    | 14,800                          | 14.3            | 10,000              | 5                 |
| AC.....               | 21.0                     | 6,380               | 6,300                      | 6,950               | 6,957              | 7,250                           | 13.7            | 11,000              | 17                |
| AC.....               |                          | 6,260 <sub>d</sub>  |                            |                     |                    |                                 |                 |                     |                   |
| AD.....               | 21.0                     | 5,390               | 5,300                      | 7,090               | 7,140              | 6,310                           | 13.7            | 11,000              | 15                |
| AD.....               |                          | 4,890 <sub>d</sub>  |                            |                     |                    |                                 |                 |                     |                   |
| AH <sub>1</sub> ..... | 21.0                     | 5,250               | 5,870                      | 6,960               | 6,120              | 5,820                           | 13.7            | 11,000              | 15                |
| AQ.....               | 21.0                     | 6,600               | 6,700                      | 6,690               | 7,070              | 7,760                           | 12.7            | 13,000              | 20                |
| AQ.....               |                          |                     |                            |                     |                    | 7,590 <sub>b</sub>              |                 |                     |                   |
| R.....                | 21.0                     | 8,930               | 9,900                      | 12,070              | 12,940             | 13,270                          | 12.3            | 14,000              | 20                |
| AJ.....               | 21.0                     | 7,750               | 8,900                      | 9,030               | 9,633              | 8,340                           | 11.8            | 15,000              | 13                |
| AJ.....               |                          |                     |                            |                     |                    | 8,500 <sub>b</sub>              |                 |                     |                   |
| AI.....               | 21.0                     | 10,000              | 9,800                      | 9,230               | 10,833             | 9,910                           | 11.8            | 15,000              | 18                |
| AE <sub>3</sub> ..... | 14.3                     | 14,800              |                            | 17,600 <sub>e</sub> |                    | 17,500                          | 11.8            | 15,000              | 6                 |
| AF <sub>2</sub> ..... | 15.4                     | 11,100              |                            | 12,666 <sub>e</sub> |                    | 13,600                          | 11.1            | 16,700              | 6                 |
| AH <sub>2</sub> ..... | 13.7                     | 5,820               |                            | 5,866 <sub>e</sub>  |                    | 4,180                           | 10.4            | 18,300              | 6                 |
| AK.....               | 21.0                     | 6,700               | 7,460                      | 8,080               | 8,535              | 8,420                           | 9.4             | 21,000              | 18                |

*a*—average 11th to 14th minutes inclusive.*b*—average 11th to 20th minutes inclusive.*c*—average 21st to 30th minutes inclusive.*d*—average for ten minutes at sea level oxygen tension at the end of the experiment; two minutes elapsed between the last reading at low oxygen tension and the beginning of this ten minute period, during which two minutes the subject was breathing sea level air outside of the rebreather.*e*—average 1st to 6th minutes inclusive.

Subnumerals in this table and in tables 2 and 4 refer to successive sections of one experiment.

TABLE 2

*Average per minute respiratory volume in cubic centimeters; subject reclining*

| SUBJECT              | INITIAL 10 MIN-UTE PERIOD |        | REBREATHING PERIOD VOLUMES |              |                     | AT REDUCED O <sub>2</sub> LEVEL |                 |                      | TIME OF REDUC-TION |
|----------------------|---------------------------|--------|----------------------------|--------------|---------------------|---------------------------------|-----------------|----------------------|--------------------|
|                      | O <sub>2</sub>            | Volume | 1-5 minutes                | 1-10 minutes | 10th minute to end  | Volume                          | O <sub>2</sub>  | Equiva-lent altitude |                    |
|                      | <i>per cent</i>           |        |                            |              |                     |                                 | <i>per cent</i> | <i>feet</i>          | <i>minutes</i>     |
| I.....               | 21.0                      | 6,590  | 7,980                      | 8,330        | 10,190              | 12,770                          | 13.7            | 11,000               | 18                 |
| J.....               | 21.0                      | 14,050 | 15,000                     | 15,180       | 16,100              | 16,100                          | 13.7            | 11,000               | 17                 |
| G <sub>1</sub> ..... | 21                        | 13,480 | 12,200                     | 13,560       | 13,550              | 14,390                          | 12.6            | 13,000               | 12                 |
| N <sub>1</sub> ..... | 21                        | 11,040 | 12,000                     | 12,200       | 12,215              | 10,960                          | 13.3            | 12,000               | 19                 |
| Q.....               | 21                        | 10,780 | 11,115                     | 11,675       |                     | 12,600                          | 13.3            | 12,000               | 8                  |
| N <sub>2</sub> ..... | 13.3                      | 10,960 | 13,300                     |              |                     | 15,270                          | 12.3            | 14,000               | 2                  |
| L.....               | 21                        | 9,050  | 10,500                     | 11,525       | 11,492              | 12,450                          | 12.3            | 14,000               | 17                 |
| F.....               | 21                        | 11,320 | 12,650                     | 13,015       | 13,030              | 12,880                          | 12.3            | 14,000               | 18                 |
| O <sub>1</sub> ..... | 21                        | 11,950 | 12,320                     | 13,860       | 14,210              | 12,520                          | 11.8            | 15,000               | 20                 |
| P.....               | 21                        | 11,980 | 11,900                     | 11,820       | 14,700              | 12,300                          | 11.1            | 16,700               | 15                 |
| O <sub>2</sub> ..... | 11.8                      | 12,520 | 12,866                     |              |                     | 13,305                          | 10.4            | 18,300               | 3                  |
| G <sub>2</sub> ..... | 12.6                      | 14,390 | 15,633                     |              |                     | 18,300                          | 10.1            | 19,000               | 3                  |
| M.....               | 21                        | 13,010 | 14,560                     | 18,840       |                     | 17,370                          | 10.1            | 19,000               | 10                 |
| H.....               | 21                        | 11,360 | 12,000                     | 12,400       | 13,155              | 16,540                          | 10.1            | 19,000               | 21                 |
| K.....               | 21                        | 8,550  | 9,200                      | 9,980        | 10,870              |                                 |                 |                      |                    |
|                      |                           |        |                            |              | 10,400 <sup>a</sup> | 12,730                          | 9.4             | 21,000               | 31                 |

<sup>a</sup>—average 21st to 31st minutes inclusive.

zero level, a volume of oxygen equal to that used by the subject during the last inspiration, entered the rebreather tank, before the water seal of the oxygen spirometer again closed. (See Larsen, '19). The subject continued this phase of the experiment using air of sea level oxygen tension until 10 to 15 minutes of normal breathing had been recorded. The oxygen valve was then quietly closed, and without any change in the resistance of the apparatus the subject began the rebreathing of the 54 liters of air in the rebreather tank. The loss of air volume due to the absorption of oxygen by the subject and the correlated absorption of carbon dioxide by the sodium hydroxide cartridge was offset by raising the movable bottom of the rebreather. By means of a scale on the rebreather the per cent of oxygen to which the air in the rebreather had been reduced could be read at any time and the equivalent altitude determined. The composition of the gas in the rebreather was also checked by analyses with a Henderson-Orsat apparatus. When the subject had reached the desired oxygen tension the oxygen valve was again opened and the oxygen tension maintained as at sea level.

The respiratory volume was read each minute from the Larsen respiration integrator (Larsen, '19). The blood pressures and pulse of the subject were taken every other minute as checks on the subject's condition.

The subjects for these experiments, drawn from the Laboratory staff and from the Air Service, were young men in good health, 20 to 30 years of age.

*Respiratory volume during the reduction of oxygen tension.* Because of the minute to minute variation in the respiratory volume readings, they have been averaged for the first five minutes of oxygen reduction, for the first ten minutes and for the remainder of the reduction period.

From tables 1 and 2 it may be seen that 23 of 29 subjects who began the reduction of oxygen tension at sea level showed an increase in the respiratory volume during the first five minutes of rebreathing, and that 25 of the 29 gave an increase during the first ten minutes. Of the four remaining cases three increased the respiratory volume during the second ten minute period.

By plotting the oxygen reduction as a straight line (the current usage of the Laboratory, verified by analyses made at different stages of rebreather tests—see Air Service Medical, '19) the approximate per cents of oxygen reached by each of the 29 men during the first five and ten minutes of rebreathing were obtained (table 3). These figures show that the increase in respiratory volume appeared very soon after the reduction of oxygen began, as 22 of the 29 subjects had not reduced the oxygen in the rebreather tank air below 18.1 per cent at the end of the fifth minute. The lowest per cent reached by any individual at the end of the fifth minute was 15.6, and the average of all cases was 18.1, equivalent to an altitude of about 4,000 feet. The average per cent reached at the end of the tenth minute was 15.2.

A comparison of the seven cases which began the reduction of oxygen in the air breathed a second time, after remaining at a level of reduced oxygen tension for some minutes, gives an increase in the respiratory volume during the transition period from one level of reduced oxygen tension to a still lower level. Considering all cases, the reduction of oxygen tension was accompanied by an increase in respiratory volume in 32 of 36 cases, regardless of the initial oxygen tension, and this increase in the respiratory volume was initiated in less than ten minutes of rebreathing.

A correlation of the rate of respiration with the respiratory volume in 20 cases indicates that there was little change in the rate of respira-

TABLE 3

*Approximate oxygen levels of subjects at the end of the 5th and 10th minutes of rebreathing*

| SUBJECT           | O <sub>2</sub> AT END<br>OF 5TH MINUTE | O <sub>2</sub> AT END<br>OF 10TH MINUTE | CHANGE IN RE-<br>SPIRATORY<br>VOLUME DURING<br>FIRST 10 MINUTES | WEIGHT<br>OF SUBJECT |
|-------------------|--|---|---|----------------------|
| Subject sitting   |  |   |   |                      |
|                   | <i>per cent</i>                        | <i>per cent</i>                         | <i>per cent</i>   | <i>pounds</i>        |
| AX.....           | 19.0                                   | 17.4                                    | 5—  | 140                  |
| AE.....           | 19.2                                   | 17.4                                    | 61+   | 175                  |
| C.....            | 18.2                                   | 15.4                                    | 74+   | 168                  |
| D.....            | 19.1                                   | 17.3                                    | 43+   | 140                  |
| AF.....           | 19.1                                   | 17.3                                    | 21+   | 133                  |
| B.....            | 18.8                                   | 16.5                                    | 75+   | 110                  |
| AB.....           | 19.0                                   | 17.4                                    | 39+   | 140                  |
| AG.....           | 18.8                                   | 16.5                                    | 36+   | 150                  |
| AA.....           | 18.1                                   | 15.3                                    | 32+   | 160                  |
| AC.....           | 19.2                                   | 17.4                                    | 9+  | 140                  |
| AD.....           | 18.6                                   | 16.2                                    | 31+   | 140                  |
| AH.....           | 18.6                                   | 16.2                                    | 33+   | 160                  |
| AQ.....           | 18.8                                   | 16.6                                    | 1+  | 150                  |
| R.....            | 18.9                                   | 16.7                                    | 35+   | 165                  |
| AJ.....           | 17.5                                   | 13.9                                    | 17+   | 165                  |
| AI.....           | 18.4                                   | 15.9                                    | 8—  | 224                  |
| AK.....           | 17.8                                   | 14.6                                    | 21+   | 163                  |
| Subject reclining |  |   |   |                      |
| I.....            | 19.0                                   | 17.1                                    | 26+   | 144                  |
| J.....            | 18.9                                   | 16.7                                    | 8+  | 110                  |
| G.....            | 17.5                                   | 13.9                                    | 7—  | 170                  |
| O.....            | 18.7                                   | 16.8                                    | 11+   | 160                  |
| P.....            | 17.6                                   | 14.3                                    | 1—  | 190                  |
| K.....            | 19.1                                   | 17.3                                    | 16+   | 160                  |
| N.....            | 19.0                                   | 17.1                                    | 11+   | 160                  |
| Q.....            | 16.1                                   | 13.2                                    | 8+  | 130                  |
| L.....            | 18.4                                   | 15.9                                    | 27+   | 160                  |
| F.....            | 18.5                                   | 16.1                                    | 15+   | 125                  |
| M.....            | 15.6                                   | 10.3                                    | 43+   | 133                  |
| H.....            | 18.4                                   | 15.9                                    | 9+  | 144                  |

tion during the first ten minutes of rebreathing (table 4). Only 3 of these 20 cases, however, failed to show an increase in the average depth of respiration during the first ten minutes. From this it is evident that the increase in respiratory volume was the result of an increase in the depth of the individual respiration.



*Respiratory volume during short exposures to constant low oxygen tension.* Table 5 summarizes the comparisons of the respiratory volumes of the subjects while breathing air of a constant oxygen tension lower than the tension of sea level air, after reaching these oxygen

TABLE 4

*Average per minute rate of respiration and average depth of respiration in cubic centimeters*

| SUBJECT               | SEA LEVEL |      | DURING REDUCTION OF O <sub>2</sub> |      |               |                   | AT REDUCED O <sub>2</sub> LEVEL |      | EQUIVALENT ALTITUDE |
|-----------------------|-----------|------|------------------------------------|------|---------------|-------------------|---------------------------------|------|---------------------|
|                       | Depth     | Rate | 1st 10 minutes                     |      | 2d 10 minutes |                   | 1st 10 minutes                  |      |                     |
|                       |           |      | Depth                              | Rate | Depth         | Rate              | Depth                           | Rate |                     |
|                       |           |      |                                    |      |               |                   |                                 |      |                     |
|                       |           |      |                                    |      |               |                   |                                 |      | feet                |
| AX.....               | 381       | 16.0 | 396                                | 14.4 |               |                   | 350                             | 16.0 | 5,000               |
| AF <sub>1</sub> ..... | 524       | 13.0 | 768                                | 12.4 | 908           | 12.0              | 925                             | 12.0 | 8,000               |
| AQ.....               | 413       | 16.0 | 446                                | 15.0 | 471           | 15.0              | 485                             | 16.0 | 13,000              |
| R.....                | 539       | 16.6 | 726                                | 16.6 | 779           | 16.6              | 799                             | 16.0 | 14,000              |
| AF <sub>2</sub> ..... |           |      | 1,005                              | 12.6 |               |                   | 1,152                           | 11.8 | 16,700              |
| AK.....               | 698       | 9.6  | 824                                | 9.8  | 888           | 9.6               | 1,005                           | 8.0  | 21,000              |
| I.....                | 366       | 18.0 | 438                                | 19.0 | 509           | 20.0              | 751                             | 17.0 | 11,000              |
| J.....                | 878       | 16.0 | 766                                | 19.8 | 894           | 18.0              | 947                             | 17.0 | 11,000              |
| G <sub>1</sub> .....  | 1,225     | 11.0 | 1,013                              | 12.4 | 1,127         | 12.0              | 1,182                           | 12.0 | 13,000              |
| O <sub>1</sub> .....  | 949       | 12.6 | 976                                | 14.2 | 1,000         | 14.2              | 963                             | 13.0 | 15,000              |
| P.....                | 798       | 15.0 | 712                                | 16.6 | 865           | 17.0              | 707                             | 17.4 | 16,700              |
| O <sub>2</sub> .....  |           |      | 989                                | 13.0 |               |                   | 924                             | 14.4 | 18,300              |
| G <sub>2</sub> .....  |           |      | 1,182                              | 13.0 |               |                   | 1,800                           | 10.3 | 19,000              |
| K.....                | 611       | 14.0 | 655                                | 15.0 | 724           | 15.0              | 909                             | 14.0 | 21,000              |
| K.....                |           |      |                                    |      | 743           | 14.0 <sub>a</sub> |                                 |      |                     |
| N <sub>1</sub> .....  | 951       | 11.6 | 1,109                              | 11.0 | 1,018         | 12.0              | 800                             | 13.7 | 12,000              |
| N <sub>2</sub> .....  |           |      | 1,023                              | 13.0 |               |                   | 1,075                           | 14.0 | 14,000              |
| L.....                | 1,005     | 9.0  | 1,152                              | 10.0 | 1,149         | 10.0              | 1,296                           | 9.6  | 14,000              |
| F.....                | 1,617     | 7.0  | 1,627                              | 8.0  | 1,150         | 8.5               | 1,842                           | 7.0  | 14,000              |
| M.....                | 813       | 16.0 | 1,008                              | 17.0 |               |                   | 1,022                           | 17.0 | 19,000              |
| H.....                | 728       | 15.6 | 689                                | 18.0 | 692           | 19.0              | 863                             | 18.0 | 19,000              |

*a*—21st to 31st minutes inclusive.

tensions by rebreathing, with the volumes moved at sea level and during the reduction by rebreathing.

Thirty-two of the 36 subjects breathed a greater volume per minute during the first ten minutes at a constant low oxygen level, than at sea level. These various oxygen tensions were equivalent to altitudes of 5000 to 21,000 feet. The four men who were held at a constant low

oxygen level for 20 minutes and the one man who was held for 30 minutes in low oxygen, each continued to breathe more air per minute throughout their sojourns in low oxygen than at sea level.

The grouping of the cases, if the respiratory volume at the reduced oxygen level is compared with the respiratory volume during the reduction of oxygen by rebreathing, is not so uniform. Seventeen of the 36

TABLE 5

*Comparison of the per minute respiratory volumes during the three phases of the experiment*

|  | SUBJECT<br>SITTING | SUBJECT<br>RECLINING | TOTAL |
|--|--------------------|----------------------|-------|
| Respiratory volume during first ten minutes at low oxygen level less than respiratory volume during reduction of oxygen but greater than sea level respiratory volume. Group 1.  | 13                 | 4                    | 17    |
| Respiratory volume during first ten minutes at low oxygen level greater than respiratory volume during reduction of oxygen and at sea level. Group 2.  | 5a                 | 10b                  | 15    |
| Respiratory volume during first ten minutes at low oxygen level less than the respiratory volume during reduction of oxygen and at sea level; respiratory volume during reduction of oxygen greater than that at sea level. Group 3. | 2                  | 1                    | 3     |
| Respiratory volume decreased throughout the experiment   | 1                  |                      |       |
| Cases.....   | 21                 | 15                   | 36    |

a—Two of these five cases gave a respiratory volume less than the respiratory volume during the reduction of oxygen, during the second ten minutes at low oxygen level.

b—One of these ten cases had exactly the same respiratory volume during the first ten minutes at the low oxygen level and the last ten minutes during the oxygen reduction.

subjects had a lower per minute respiratory volume during the first ten minutes in low oxygen of a constant tension than during the period of rebreathing which preceded this exposure. Of the five men who were held in low oxygen for more than ten minutes four gave a lower respiratory volume for the second ten minutes of the exposure than for the first ten minutes.

The average depth of respiration of the subjects during these exposures to constant low oxygen was greater than at sea level in 15 of 20 cases. The respiratory rate was the same as or lower than the sea level rate in 11 of 20 cases.

#### DISCUSSION

The increase in respiratory volume during the reduction of the oxygen tension found in these experiments agrees with previous work of other writers who reduced the oxygen tension of the air breathed by their subjects at a fairly rapid rate. The increase here discussed can not be ascribed to the resistance of the apparatus (which was practically negligible) as the subject respired through the apparatus during all phases of the experiment, i.e., during the sea level normals as well as during the reduction by rebreathing and during the exposures at low oxygen levels; nor is it the result of an accumulation of carbon dioxide in the rebreather, as check analyses of the gas in the rebreather were made with the Henderson-Orsat gas apparatus. This increase in respiration does, however, accompany the reduction of oxygen tension and begins very early in the reduction phase of the experiment. Haldane, Meakins and Priestley ('19) offer an explanation of this response by stating that the first result of the diminution of the percentage of oxygen is an increase in the depth of respiration owing to a lowering of the threshold of exciting value of carbon dioxide. This explanation if applied to the present experiments would call for a change in the carbon dioxide threshold very early in the rebreathing reduction of oxygen as the tables show that most of the subjects had responded with an increase in respiration by the end of the fifth minute and to an average oxygen per cent of 18.1. Although the individual minute to minute data for respiratory volume can not be given here, they showed when plotted as curves, a distinct upward trend in every case after the first or second minute of rebreathing, suggesting that the actual increase in respiratory volume began earlier than the fifth minute and at an oxygen per cent higher than 18.1. If the response to reduction of oxygen tension by increase in respiratory volume began above 18.1 oxygen it might have been so small as to be easily masked by other factors and escape notice on the rebreather. That there is a progressive increase in the magnitude of this increase in volume response as the reduction of the oxygen progresses is suggested by a comparison of the per minute volume during the first ten minutes of the rebreathing reduction with the per minute respiratory volume during the second

ten minutes of rebreathing, the volume in the second period being higher in the majority of cases.

Considering the average of 18.1 per cent oxygen as the point at which a definite increase in respiratory volume was found, the responses of 29 subjects examined came earlier in the rebreathing reduction, i.e., at a higher oxygen per cent, than in the cases given by Schneider (l.c.) but at almost exactly the same level as that given by Lutz and Schneider (l.c.) for the onset of increased respiratory volume in their low pressure chamber and Dreyer nitrogen apparatus experiments. It is interesting to note that they found the alveolar carbon dioxide tension definitely lowered at 656 mm. of mercury pressure, which is approximately equivalent to 18.1 per cent oxygen.

The larger respiratory volume moved per minute by subjects during short exposures to low oxygen after a rather rapid reduction in oxygen tension by rebreathing, as compared with their sea level per minute respiratory volumes might be expected in the light of the increase in respiratory volume at 18.1 per cent oxygen. The fall in per minute respiratory volume during the first ten minutes of these exposures to constant low oxygen, to a volume lower than that moved during the reduction of oxygen by rebreathing, in 17 of 36 cases, presents an interesting example of rapid compensation to low oxygen. Lutz and Schneider (l.c.) found a similar fall in the per minute respiratory volume in 9 of 14 cases held at 20,000 feet in the low pressure chamber, and state that they believe this fall in respiratory volume to represent a temporary improvement in the subject's condition. That this fall in respiratory volume does indicate more or less compensation to the new conditions of low oxygen and an improvement in general condition of the subject is suggested in the present experiments by two comparisons.

If the 36 subjects are divided with regard to the per cent of oxygen in the air in which they were held during the exposures to constant low oxygen, i.e., the line of equivalent altitude, 10 of the 33 cases were held at tensions equivalent to altitudes varying from 5000 to 10,000 feet inclusive. Of these ten cases, eight had lower respiratory volumes during the first ten minutes at constant low oxygen levels than during the reduction of oxygen; and the other two cases, although maintaining a higher per minute volume during the first ten minutes at the new oxygen level, decreased their per minute volumes during the second ten minutes of the exposure to constant low oxygen, to volumes lower than those breathed during the reduction of oxygen. These cases held at comparative low altitudes, or comparatively high oxygen per cents,



constitute half the cases in group 1 of table 5. If the per cent of oxygen in the air breathed during these exposures to low oxygen may be taken as a gross index of the severity of the conditions to which the subjects were attempting compensations, the lower the oxygen per cent and the higher the equivalent altitude, the greater the task of adjustment. That the subjects exposed to the less severe conditions did show this fall in respiratory volume during the first ten minutes in constant low oxygen, does therefore favor the view that the fall in respiratory volume (pulse and blood pressure remaining good) is associated with compensations to reduced oxygen tension, which enable the body to maintain itself without so great a per minute respiratory volume.

As shown by the blood pressure and pulse records, subject K was approaching a collapse during his sojourn at 21,000 feet, i.e., he was not maintaining nor improving his condition at that level of low oxygen. His per minute respiratory volume did not fall during the ten minutes he was held at 21,000 feet, but on the contrary continued to increase above the volume breathed during the first, second and third ten minute periods of rebreathing. This case gives the response in respiratory volume of a subject exposed to conditions of low oxygen obviously too severe for his compensatory complex at the time of this particular experiment. The response of subject K also indicates that the fall in respiratory volume of subjects under less severe conditions was correlated with advantageous compensations.

Although the respiratory volume during exposures to constant low oxygen tension was greater than the respiratory volume at sea level, the return to sea level air was accompanied by a very prompt return to the sea level respiratory volume. To check by quantitative methods the general observation made on all subjects, subjects AA, AC and AD were carried through a fourth phase of experimentation. After completing the exposure to low oxygen the valves were opened and the subject allowed to breathe pure, unmixed, sea level air through the rebreather tubes for two minutes. This change to sea level was instantaneous, and it was presumed that the lungs of the subject were fairly well ventilated by breathing outside air for two minutes. At the end of two minutes the rebreather valves were again closed and the subject's per minute respiratory volume while breathing pure sea level air was obtained as at the beginning of the experiment, for an additional ten minutes.

The average per minute respiratory volume of subject AA returned to within 250 cc. of his original sea level respiratory volume, during

this ten minute after-period at sea level, although his increase in respiratory volume at the low oxygen level was 4520 cc. The respiratory volume of subject AC fell during the ten minute after-period at sea level 120 cc., and that of subject AD, 500 cc. below the sea level respiratory volume taken at the beginning of the experiments. The increases in respiratory volumes at the low oxygen levels were 870 cc. and 920 cc. respectively for these two men.

One correlation of the position of the subject during the test with the increase in respiratory volume during the reduction of oxygen by rebreathing seems worthy of note. The per cent of increase in the respiratory volume at the end of the tenth minute of rebreathing was higher for the sitting subjects than for the reclining subjects. As this may be a function of the relative metabolic rate in the two positions, it may be added that Emmes and Riche ('11) found the rate of metabolism in sitting subjects to be 7.6 per cent higher than in reclining subjects, as shown by their oxygen consumption.

#### SUMMARY

1. The respiratory volumes of 29 men during the reduction of oxygen tension by rebreathing and during short exposures to constant low oxygen tension following the period of rebreathing were studied in connection with the sea level respiratory volumes.

2. An increase in the respiratory volume was noted at the end of the fifth minute of rebreathing, at an average of 18.1 per cent oxygen (approximately equivalent to 4000 feet altitude) in 23 of the 29 subjects.

3. The minute to minute respiratory volumes suggest that this increase may occur even earlier in the rebreathing period.

4. The respiratory volume during the first ten minutes of the exposure to constant low oxygen tension, varying from 5000 to 21,000 feet equivalent altitude, was greater than the sea level volume in 32 of the 36 cases.

5. The respiratory volume of 17 of the 36 cases fell during the first ten minutes of the exposure to constant low oxygen tension to a volume lower than that moved during the reduction of oxygen by rebreathing, although still greater than the sea level respiratory volume. These 17 cases include all but one of the cases held at oxygen levels equivalent to 10,000 feet or less.

6. This fall in respiratory volume during the first ten minutes of exposure to constant low oxygen tension was correlated apparently with compensations to low oxygen advantageous to the subject.

7. The return to sea level oxygen tension was followed by a prompt return to the sea level respiratory volume.

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## ALVEOLAR AIR AND RESPIRATORY VOLUME AT LOW OXYGEN TENSIONS

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Modern warfare has not only created a need for quick ascents to high altitudes for brief periods, but has made it necessary frequently for pilots and observers in reconnaissance to remain one or two hours at 15,000 feet or higher. In the light of data recently published (1) from this laboratory, it seems clear that during exposures of an hour or more relatively permanent factors may relieve the more temporary means of compensation which come into play when the ascent is made at the rate of 1000 feet per minute. In connection with the experimental work along these lines there was an opportunity to determine the alveolar air and respiratory volume under conditions which simulated, so far as time and pressure are concerned, an ascent in an airplane to 18,000 or 20,000 feet. Many of the subjects were maintained at these levels for periods varying between 20 and 127 minutes, during which the alveolar air or the respiratory volume was followed.

Few observations have been made under the conditions mentioned above. Haldane and Poulton (2) report great hyperpnoea when the oxygen in the inspired air is reduced from 10 per cent to about 5 per cent in a few minutes. When the reduction was made more slowly, from about 12 per cent to 9 per cent in 30 minutes, there was no noticeable hyperpnoea. The alveolar air fell to values between 3.9 and 4.3 per cent. Schneider (3) reported an increase in lung ventilation, beginning as soon as the oxygen per cent had been reduced by the re-breathing method to 16 or 14 per cent, when the rate of reduction was about equivalent to an ascent of 1000 feet per minute. Lutz (4) has reported that at this rate of ascent the onset of increased breathing may come as early as 656 mm. (4000 feet) in the low pressure chamber.

Many investigators have shown that the lung ventilation is increased at low oxygen levels when the low level is reached after some delay. Loewy (5) reported that the volume of air breathed began to increase



at a reduction pressure of 580 mm. (7000 feet). Loewy and Zuntz (6) found a 4 per cent increase in ventilation when the pressure was reduced to 448 mm. (13,800 feet). Boycott and Haldane (7) found that when the atmospheric pressure was diminished, the alveolar carbon dioxide remained constant until the air pressure fell to 550 mm. (14 per cent oxygen at 760 mm.), or until the alveolar oxygen tension was lowered to about 62 mm. At lower air pressures the carbon dioxide tension fell with increasing rapidity. In these exposures the reduction of the barometric pressure to 350 mm. covered a period of two hours or longer.

Alveolar air tensions found by observers on mountains of moderate height show changes similar to those found in low pressure chamber work. Haldane and Priestley (8) report their alveolar carbon dioxide tensions taken at Oxford and on Ben Nevis (4406 feet). That of J. S. H. fell from 39.6 mm. to 37.0 mm., while that of J. G. P. dropped from 44.5 mm. to 42.4 mm. This change of a little more than 2 mm. was not ascribed to low pressure but to the effects of fatigue, since the subject walked up the mountain. Ward (9) compared his alveolar air values at London, Zermatt (5315 feet) and Monte Rosa (14,965 feet). The entire ascent was made during a period of days, during which his alveolar carbon dioxide tension fell from 37.7 mm. at London, to 34.2 mm. at Zermatt, and then to 28.5 mm. at the summit of Monte Rosa. Such a fall means a marked increase in ventilation. Douglas, Haldane, Henderson and Schneider (10) found a fall of alveolar carbon dioxide from about 40 mm. to 27 mm. The ascent from Manitou (6485 feet) to the summit of Pike's Peak (14,110 feet) was made on the railroad in about an hour and a half. One subject, E. C. S., showed a fall to 33.5 mm. just after arriving; another, Y. H., gave 33.4 mm. Determinations were not made on C. G. D. and J. S. H. until nearly an hour after their arrival. The former gave 32.2 mm., the latter 31.6 mm. FitzGerald (11) determined the alveolar air on persons living permanently at various altitudes up to 14,000 feet and found the carbon dioxide tension already lowered at 700 mm. (2200 feet). Hasselbalch and Lindhard (12), however, report that in steel chamber experiments in which the barometer was lowered from 756 mm. to 541 mm. (10,000 feet) in three or four days, little change in rate or volume of breathing occurred. Mosso (13) did not find any clear-cut increase in ventilation at high altitudes.

It cannot be doubted that there is a lowering of the alveolar oxygen and carbon dioxide tensions at reduced atmospheric pressures. A lowering of the carbon dioxide tension, other things being equal, signifies increased ventilation. However, the changes in the alveolar air and

ventilation which occur when the individual is subjected to conditions comparable, so far as pressure is concerned, to rapid airplane ascents and reconnaissance have not been clearly described.

#### METHOD

Low oxygen tension was produced by two methods, first by reducing the barometric pressure in a low pressure chamber, and second by the rebreathing method in which the subject gradually reduces the oxygen in a given volume of air, the carbon dioxide being removed by sodium hydroxide. The majority of the experiments reported in this paper were conducted in the low pressure chamber. A number were made with the rebreather for comparison.

The construction of the chamber is described elsewhere (14). While reduction was going on or while a reduced pressure was being held, sufficient ventilation could be maintained to prevent an accumulation of carbon dioxide or oxygen in the respired air. The rate of reduction was uniform and equivalent to an ascent of 1000 feet per minute.

Alveolar air samples were taken in the following manner. After a period of normal breathing ending with an expiration, a quick forced expiration was made into a Henderson alveolar air sampling tube (15). This is essentially a modification of the Haldane-Priestley method of taking an expiratory sample, since the last 75 cc. of the forced expiration remains in the tube. Samples for analysis were drawn directly into a Henderson-Orsat analyzer (16) in which a 1 per cent solution of sulfuric acid in 50 per cent ethyl alcohol was substituted for the 1 per cent acidified water ordinarily used. It was found that such a solution hastened drainage and prevented droplets from standing on the inside of the gas burette, thereby increasing the accuracy as well as the rapidity with which an analysis could be made. Although the method used for taking air samples probably gives slightly higher oxygen and carbon dioxide than more elaborate indirect methods (17), it is pointed out by Pearce (18), (19) that the Haldane-Priestley method is less likely to give such high results for the carbon dioxide than for the oxygen. Certain practical considerations influenced the choice of alveolar air sampling; first, the sample had to be taken quickly while the pressure was being reduced; second, a technique requiring the measurement of air volumes by ordinary spirometers is subject to error in low pressure chamber work when the pressure is being changed. The volume of breathing was determined by two methods. Usually

a continuous record of the volume breathed per minute was obtained. In the first method the subject wore a part of an American Tissot gas mask and inspired through an American light meter no. 5. This meter had a resistance of 3 inches of water to a 20 liter per minute flow. Controls, made at sea level for periods up to 111 minutes, showed no effect

TABLE 1

*Alveolar air tensions in men during reduction of pressure to 352 mm. (20,000 feet) at a rate equivalent to 1000 feet per minute*

|         | 760 MM.        |                 | 656 MM.        |                 | 560 MM.        |                 | 480 MM.        |                 | 410 MM.        |                 | 352 MM.        |                 |
|---------|----------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|
|         | O <sub>2</sub> | CO <sub>2</sub> | O <sub>2</sub> | CO <sub>2</sub> | O <sub>2</sub> | CO <sub>2</sub> | O <sub>2</sub> | CO <sub>2</sub> | O <sub>2</sub> | CO <sub>2</sub> | O <sub>2</sub> | CO <sub>2</sub> |
| 1       | 109.1          | 40.3            | 87.1           | 37.7            | 68.2           | 36.4            | 60.2           | 31.7            | 43.6           | 34.5            | 39.9           | 28.0            |
| 2       | 107.0          | 36.0            | 93.8           | 32.5            |                |                 | 56.7           | 33.3            | 45.7           | 30.6            | 27.8           | 25.9            |
| 3       | 102.8          | 40.1            |                |                 |                |                 | 50.2           | 32.6            |                |                 | 38.4           | 26.2            |
| 4       | 105.6          | 38.5            | 91.4           | 33.4            | 73.8           | 33.5            | 58.4           | 34.5            |                |                 | 36.0           | 31.2            |
| 5       | 109.1          | 34.9            |                |                 | 67.2           | 36.0            | 45.4           | 33.8            | 40.7           | 29.2            | 33.9           | 27.4            |
| 6       | 99.1           | 36.2            |                |                 | 64.6           | 32.3            | 49.3           | 31.3            | 40.7           | 27.9            |                |                 |
| 7       | 97.0           | 41.6            | 81.6           | 37.7            | 69.3           | 36.2            |                |                 | 43.6           | 27.7            | 35.1           | 24.7            |
| 8       | 109.9          | 36.8            |                |                 |                |                 |                |                 | 37.4           | 33.8            |                |                 |
| 9       | 102.0          | 39.5            | 76.7           | 38.0            | 60.0           | 39.6            |                |                 | 38.5           | 34.8            | 30.5           | 30.5            |
| 10      | 87.8           | 44.8            | 70.0           | 39.4            | 58.5           | 35.0            | 47.2           | 33.3            | 39.6           | 30.2            | 32.9           | 26.0            |
| 11      | 104.9          | 41.6            | 85.2           | 40.0            | 63.6           | 37.4            | 56.8           | 34.2            | 41.4           | 30.3            | 36.0           | 27.6            |
| 12      | 103.4          | 39.5            | 92.5           | 32.5            |                |                 | 56.8           | 31.5            | 50.1           | 28.6            | 36.3           | 29.3            |
| 13      | 98.5           | 45.1            |                |                 | 62.6           | 41.2            | 48.9           | 38.5            | 43.2           | 35.6            | 34.2           | 39.8            |
| 14      | 109.9          | 38.4            | 86.4           | 37.1            | 72.9           | 35.8            | 54.1           | 35.1            | 42.8           | 35.6            | 33.6           | 33.4            |
| 15      | 104.1          | 41.3            | 83.4           | 38.9            | 70.8           | 35.6            | 57.6           | 35.9            | 45.0           | 34.1            | 34.8           | 32.2            |
| 16      | 94.2           | 40.5            | 71.8           | 37.9            | 58.5           | 35.1            |                |                 | 36.6           | 32.2            |                |                 |
| 17      | 105.6          | 40.8            | 86.4           | 39.4            |                |                 |                |                 | 40.7           | 32.7            | 31.7           | 33.3            |
| 18      | 107.0          | 37.8            |                |                 | 58.5           | 39.6            | 50.6           | 34.8            | 45.7           | 30.4            |                |                 |
| 19      | 103.4          | 39.9            |                |                 | 68.8           | 36.4            | 53.3           | 33.8            |                |                 | 37.5           | 31.1            |
| 20      | 104.9          | 37.4            |                |                 | 71.4           | 32.9            | 57.1           | 29.0            | 48.6           | 28.1            | 36.6           | 28.0            |
| 21      | 93.5           | 44.0            | 79.7           | 42.2            | 66.7           | 36.8            |                |                 |                |                 | 36.3           | 32.3            |
| 22      | 107.0          | 39.6            | 91.4           | 33.4            | 71.8           | 33.4            | 52.4           | 33.2            | 43.9           | 27.9            | 33.9           | 28.3            |
| 23      | 107.8          | 38.7            |                |                 |                |                 | 52.4           | 33.2            | 41.8           | 32.4            | 38.1           | 30.4            |
| 24      | 104.9          | 39.5            | 80.4           | 35.8            | 62.1           | 37.6            |                |                 | 41.4           | 29.2            | 33.2           | 35.6            |
| Average | 103.2          | 39.7            | 83.7           | 37.0            | 66.0           | 36.2            | 53.3           | 33.6            | 42.6           | 31.3            | 34.8           | 30.0            |

either on the volume per minute or the rate. In the second method a spirometer devised by Larsen (20) was used. In this apparatus the resistance is negligible. It consists of a calibrated spirometer from which the subject inspires through a mouth-piece, a clip being placed on the nose. Each expiration operates an electric valve which opens the spirometer to the low pressure chamber during the expiration and

thus prevents a difference in air pressure inside and outside of the spirometer, which would make the readings valueless.

*Alveolar air changes during gradual decrease in barometric pressure.* In twenty-four cases in which the subjects were taken to 352 mm. (20,000 feet) at a rate equivalent to 1000 feet per minute, the alveolar tensions were determined for 760 mm., 656 mm., 560 mm., 480 mm., 410 mm. and 352 mm., or sea level, 4000 feet, 8000 feet, 12,000 feet, 16,000 feet and 20,000 feet respectively. The alveolar air tensions are given in table 1. The average figures shown at the bottom of the table are shown graphically in figure 1. The average alveolar oxygen tension at sea level

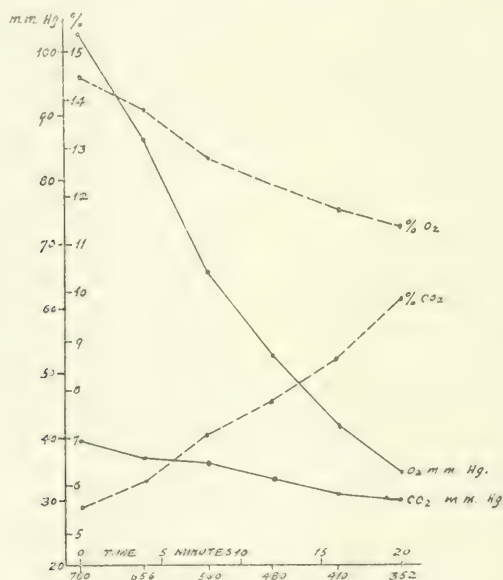


Fig. 1. Average alveolar air changes in 24 men taken to 352 mm. (20,000 feet) in a low pressure chamber at a rate equivalent to 1000 feet a minute.

was 103 mm., and for 352 mm. it was 34.8 mm., thus showing a fall of about 68 mm. or 66 per cent. The maximum fall was from 107 mm. to 27.8 mm., or 79.2 mm. (74 per cent). The minimum change was from 87.8 mm. to 32.9 mm., or 54.9 mm. (63 per cent). The average alveolar carbon dioxide tension for 760 mm. was 39.6 mm., and for 352 mm. it was 30 mm., thus showing a fall of 9.6 mm. or 24 per cent. The maximum fall was from 44.8 mm. to 26.0 mm. or 18.8 mm. (42 per cent). The minimum change was from 39.5 mm. to 35.6 mm., or 3.9 mm. (10 per cent).



These data show a fall in both oxygen and carbon dioxide tensions present at 656 mm. (4000 feet). The fall in carbon dioxide tension was a little more than 2 mm. at this pressure, which corresponds to the drop of a little more than 2 mm. in the alveolar carbon dioxide determinations of Haldane and Priestley (8) taken at Oxford and on Ben Nevis (4406 feet) and which they did not ascribe to low oxygen. The lowered carbon dioxide indicates that an increase in lung ventilation had already begun at this low altitude, although it is difficult to determine this slight increase by measuring the volume of air breathed per minute, as will be seen from data presented later in this paper. This



Fig. 2. Alveolar air tensions of J. B. D. taken to 352 mm. (20,000 feet) in a low pressure chamber. Dotted lines on 8/14/18 at a rate of 1000 feet a minute. Solid lines on 8/21/18 at a rate of 500 feet a minute. The carbon dioxide tension of 8/14/18 is plotted 1 mm. lower than the actual value.

early response to low oxygen, which has been quickly produced, is interesting when it is compared with FitzGerald's work (10), which shows a fall in alveolar carbon dioxide present in men living permanently at 700 mm. (2200 feet).

Individual curves show the early fall in alveolar carbon dioxide tension just as strikingly as the average curves. J. B. D. (fig. 2) was taken to 352 mm. on two different days a week apart. On the first

TABLE 2  
*Alveolar air tensions. Repeated rapid reductions in pressure in the low pressure chamber*

| TIME MINUTES.....                              | 0     | 8    | 15   | 20   | 28    | 35   | 40   | 48   | 55   | 60   | 68   | 75   | 80    | 88   | 95   | 100  | 108  | 115   |
|--|-------|------|------|------|-------|------|------|------|------|------|------|------|-------|------|------|------|------|-------|
| Barometer mm. Hg.....                          | 760   | 560  | 428  | 428  | 572   | 700  | 700  | 556  | 428  | 428  | 572  | 700  | 700   | 556  | 428  | 428  | 585  | 760   |
| E. L. B. 5/14/18 { O <sub>2</sub> tension..... | 102.0 | 65.7 | 34.6 | 34.1 | 162.6 | 83.0 | 88.5 | 61.0 | 44.8 | 43.8 | 61.9 | 81.1 | 188.5 | 62.0 | 41.7 | 39.6 | 62.5 | 100.0 |
| CO <sub>2</sub> tension.....                   | 40.3  | 35.4 | 32.6 | 32.9 | 37.2  | 36.5 | 36.6 | 35.2 | 31.0 | 32.0 | 35.6 | 36.1 | 137.1 | 34.7 | 33.8 | 32.5 | 35.4 | 37.4  |
| C. N. 5/14/18 { O <sub>2</sub> tension.....    | 104.4 | 60.7 | 39.7 | 41.0 | 52.0  | 90.5 | 87.0 | 62.0 | 42.5 | 36.8 | 70.0 | 84.5 | 73.0  | 41.3 | 35.3 | 65.2 | 98.4 |       |
| CO <sub>2</sub> tension.....                   | 41.7  | 39.7 | 40.3 | 37.4 | 44.2  | 43.5 | 43.5 | 42.3 | 38.2 | 39.4 | 39.8 | 43.5 | 39.9  | 37.0 | 40.5 | 42.0 | 41.2 |       |
| I. M. 5/13/18 { O <sub>2</sub> tension.....    | 106.0 | 69.5 | 49.2 | 52.5 | 75.5  | 93.0 | 99.0 | 44.4 | 48.3 | 70.7 | 99.5 | 91.7 | 70.6  | 46.9 | 48.0 | 65.5 | 95.0 |       |
| CO <sub>2</sub> tension.....                   | 41.0  | 34.8 | 31.1 | 27.3 | 32.1  | 34.8 | 33.2 | 33.8 | 29.6 | 32.2 | 35.2 | 37.3 | 35.3  | 32.2 | 28.7 | 33.5 | 40.5 |       |
| TIME MINUTES.....                              | 0     | 10   | 15   | 21   | 28    | 35   | 40   | 48   | 55   | 65   | 85   | 93   | 102   |      |      |      |      |       |
| Barometer mm. Hg.....                          | 760   | 510  | 425  | 430  | 595   | 700  | 700  | 510  | 425  | 425  | 425  | 570  | 760   |      |      |      |      |       |
| A. F. H. 5/21/18 { O <sub>2</sub> tension..... | 106.4 | 59.8 | 47.0 | 43.1 | 65.2  | 86.4 | 91.0 | 58.5 | 41.2 | 38.6 | 37.5 | 58.2 | 93.5  |      |      |      |      |       |
| CO <sub>2</sub> tension.....                   | 38.9  | 31.2 | 30.6 | 31.7 | 35.2  | 35.2 | 34.8 | 33.8 | 31.6 | 30.1 | 27.9 | 34.3 | 36.7  |      |      |      |      |       |

day the pressure was reduced at a rate equivalent to 1000 feet per minute, and the reduction was made in 20 minutes. Alveolar air samples were taken every 4 minutes. On the second day the pressure was reduced half as fast and samples were taken every 5 minutes. On the first day the oxygen fell from 105.5 mm. to 37 mm., 68.5 mm. or 65 per cent. The carbon dioxide fell from 42 mm. to 28.2 mm., 13.8 mm. or 32.9 per cent. On the second day the fall in oxygen was 67.5 per cent, and in carbon dioxide it was 32.7 per cent. On both days a definite fall in carbon dioxide tension (2 to 2.5 mm.) was present at 656 mm. (4000 feet).

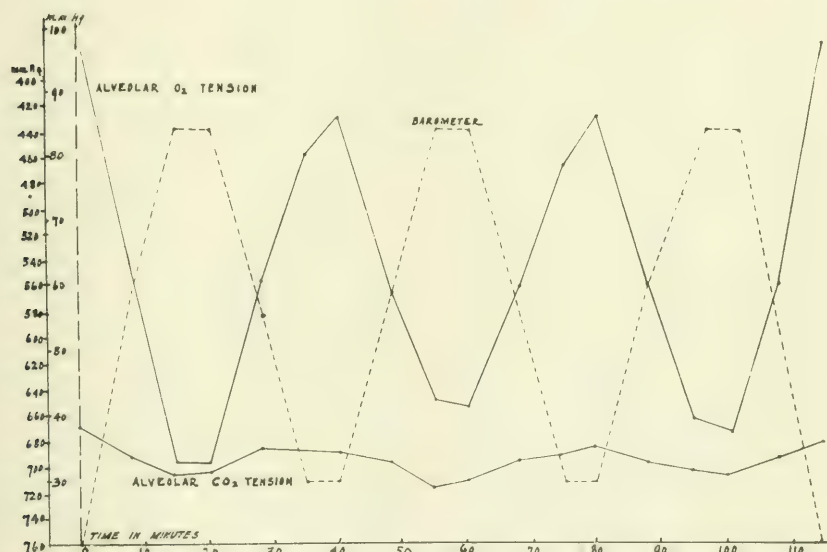


Fig. 3. Alveolar air tensions of E. L. B., 5/14/18, taken three times to 428 mm. (15,000 feet) at a rate of 1000 feet a minute.

That both the alveolar oxygen and carbon dioxide tensions are quickly lowered and quickly returned with rapid reduction, brief exposure, and rapid increase of barometric pressure is shown in four experiments in table 2, the first of which, E. L. B., is seen graphically in figure 3. In these cases the barometer was lowered to 428 mm. (15,000 feet) in 15 minutes, held at that level for 5 minutes, then increased to 700 mm. (2200 feet) at the same rate and held at this new level for 5 minutes, when the ascent was resumed. The reduction of pressure was repeated three times in succession. The fact that the carbon dioxide tension

TABLE 3

| NAME         | REDBREATHER                                    |               |              |                   |                               | LOW PRESSURE CHAMBER                           |               |              |                   |                 |                                 |  |
|--------------|--|---------------|--------------|-------------------|-------------------------------|--|---------------|--------------|-------------------|-----------------|---------------------------------|--|
|              | Date   | Alveolar air  |              | Per cent decrease | Final per cent O <sub>2</sub> | Date   | Alveolar air  |              | Per cent decrease | Final barometer | O <sub>2</sub> partial pressure |  |
|              |  | 760           | Final        |                   |                               |  | 760           | Final        |                   |                 |                                 |  |
| I. M.....    | 4/10/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 100.2<br>38.8 | 41.8<br>32.6 | 58.1<br>16.0      | 9.7                           | 4/17/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 103.2<br>40.0 | 37.1<br>30.4 | 64.0<br>24.0      | 350             | 73.5                            |  |
| W. O. K..... | 4/22/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 97.5<br>43.1  | 36.1<br>34.5 | 63.0<br>19.9      | 9.0                           | 4/27/18 {<br>O<br>CO <sub>2</sub>              | 104.5<br>39.5 | 32.1<br>30.3 | 69.0<br>29.0      | 325             | 68.0                            |  |
| H. F. P..... | 4/20/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 102.3<br>40.4 | 38.6<br>32.3 | 62.1<br>20.1      | 9.8                           | 4/30/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 107.5<br>38.8 | 37.9<br>30.2 | 64.8<br>22.2      | 368             | 74.5                            |  |
| S. M. J..... | 4/30/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 97.5<br>45.2  | 37.0<br>34.1 | 62.0<br>24.8      | 9.0                           | 5/1/18 {<br>O <sub>2</sub><br>CO <sub>2</sub>  | 107.0<br>37.6 | 33.6<br>26.7 | 69.5<br>29.0      | 325             | 68.0                            |  |
| R. M. B..... | 4/18/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 108.0<br>43.0 | 37.5<br>39.4 | 65.2<br>8.4       | 10.3                          | 4/19/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 105.2<br>39.7 | 33.0<br>34.2 | 68.6<br>13.8      | 365             | 76.5                            |  |
| E. L. B..... | 5/14/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 105.0<br>38.3 | 34.3<br>35.2 | 67.4<br>8.1       | 10.3                          | 5/15/18 {<br>O <sub>2</sub><br>CO <sub>2</sub> | 102.0<br>40.3 | 31.6<br>32.6 | 66.0<br>19.1      | 365             | 76.5                            |  |



changed so readily with the pressure indicates that there was little permanent disturbance in the carbon dioxide level, although it should be noted that the carbon dioxide tension did not entirely return to the starting level each time that 700 mm. was reached.

*Alveolar air in the rebreathing and in the low pressure chamber methods.* In six men the alveolar air tensions in the low pressure chamber and in the rebreathing method were compared. The subjects were first taken on the rebreather. Alveolar air samples were taken by means of a special three-way mouth-piece, about every 4 minutes during the run,

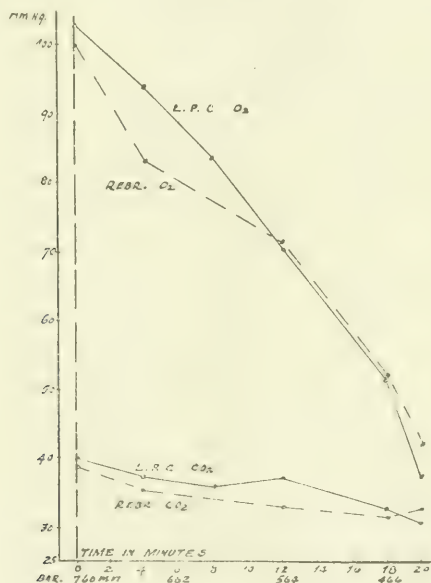


Fig. 4. Alveolar air tensions of R. M. B., 4/18/18, taken on the rebreather to 10.3 per cent oxygen (dotted line). 4/19/18, taken to 365 mm. in the low pressure chamber at the same rate.

with a final sample just as the experiment was stopped. From the percentage of oxygen reached the corresponding barometer was computed and later the subjects were taken in the low pressure chamber to the calculated barometric pressure at a rate corresponding exactly to the reduction of oxygen tension by the rebreathing method. Alveolar air samples were again taken at corresponding times.

The data presented in table 3 and the case of R. M. B. in figure 4 show that the two methods of producing low oxygen partial pressure

are essentially the same so far as the effects on alveolar tension are concerned. In one case the alveolar air tensions were determined in a man taken to 10 per cent oxygen in 20 minutes by the Dreyer nitrogen dilution method (21). In this case the oxygen tension fell from 102.5 mm. to 32.8 mm., and the carbon dioxide pressure from 38.7 mm. to 33.3 mm. Both of these methods emphasize that barometric pressure in itself is not a causative factor in the responses to low oxygen tension except as a means of producing low oxygen tension.

*Alveolar air during maintained low oxygen pressure.* The alveolar air tensions immediately on ascending to 428 or 380 mm. (15,000 or 18,000 feet) indicate that an increase in ventilation has occurred. There is no doubt that if men stayed long enough at these altitudes they would become mountain-sick. But they may tolerate these altitudes for one or two hours and feel little or no illeffects after the flight. The course of the alveolar tensions during periods of low oxygen level of from 30 to 120 minutes was followed in the low pressure chamber in fourteen cases, by taking alveolar samples every 5 or 10 minutes.

In five subjects taken to 428 mm. (15,000 feet) in 15 minutes and maintained at that pressure for periods varying from 30 to 90 minutes, four showed a fall in carbon dioxide tension during the ascent present at 560 mm. (8000 feet). This fall varied from 1.6 mm. to 4.3 mm. and averaged 3.1 mm. All showed a drop in carbon dioxide tension which averaged 6.7 mm. (7.3 per cent) after having been at 428 mm. for 5 minutes. The average alveolar oxygen tension had fallen from 100.4 mm. to 41.5 mm. at this time, an average drop of 58.9 mm. or 58.6 per cent. In four cases the oxygen tension maintained its low level as the experiment proceeded. In these cases the carbon dioxide tension maintained its new level with little variation. In one case, W. O. K., which lasted 90 minutes, there was a definite rise in oxygen tension during the last 40 minutes, and the carbon dioxide tension fell to 24.4 mm. toward the end. It was evident from these experiments that these men tolerated 428 mm. with little discomfort, since the respiration increased moderately and maintained its new level.

That the men at 428 mm. were not under stress is shown also by the normal alveolar air taken within 20 minutes after 760 mm. had been reached. The carbon dioxide tension of W. O. K. returned only to 34.6 mm. while the others showed a complete recovery to the former tension.

Nine men were taken to 380 mm. (18,000 feet) and maintained at that level for from 50 to 120 minutes. At this altitude more profound changes than those shown at 15,000 feet were expected. The data



obtained from this series are presented in table 4. Since alveolar air samples were usually taken every 5 minutes at the desired level, the figures presented represent, for the most part, the average of two samples taken during the period indicated in the table. The average oxygen tension at 760 mm. was 104 mm. It fell to 36.4 mm., 67.6 mm. or 65

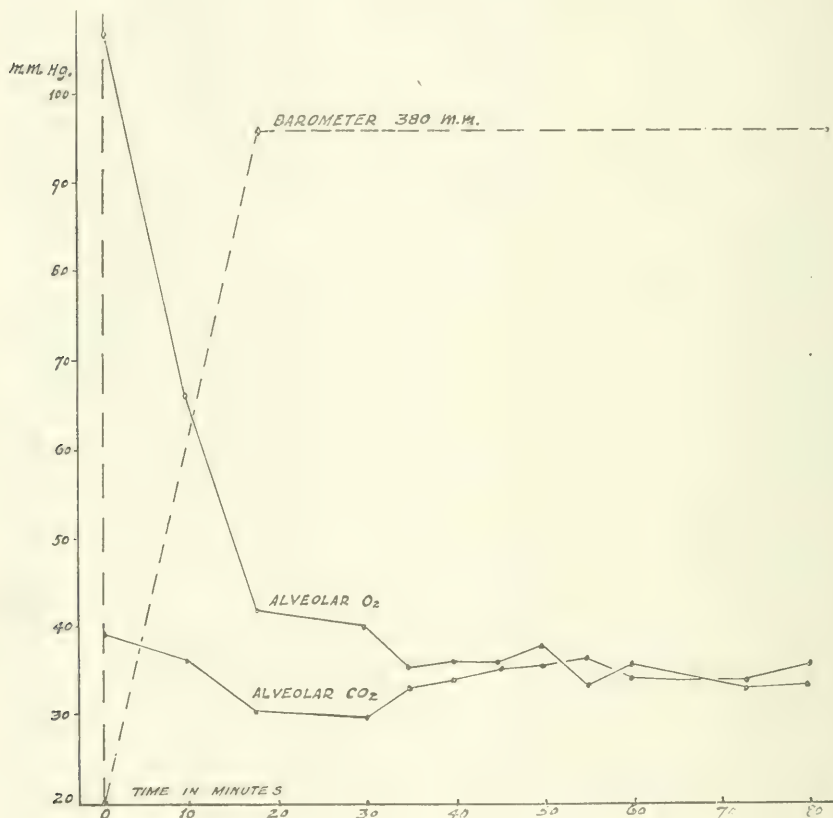


Fig. 5. Alveolar tensions of N. E. F., 6/7/18, taken to 380 mm. (18,000 feet) in 18 minutes and maintained at that level. Note the low carbon dioxide tension just after the low barometric pressure was attained.

per cent, before the subjects had been at 380 mm. for 10 minutes. The average figure had fallen to 34.9 mm. 20 to 30 minutes later. The average carbon dioxide tension at 760 mm. was 38.7 mm. It fell to 30.4 mm., 8.3 mm. or 21.4 per cent, shortly after the subjects had reached



380 mm., but before 10 minutes at that altitude. Twenty to 30 minutes later the average figure had risen to 31. The more profound the effects of 380 mm. over 428 mm. are shown in the percentage of decrease in the alveolar air pressures. For the oxygen it was 58.6 per cent and 65 per cent for 428 mm. and 380 mm. respectively. As might be expected, the more striking effect is shown in the carbon dioxide. The percentage decrease was 17.3 and 21.4 for 428 mm. and 380 mm. respectively.

The alveolar carbon dioxide pressures showed three general types of curves during these experiments. In one case the pressure fell and continued to fall, B. M. L. In two cases it fell and maintained its low level, R. S. S. and A. W. L. In six cases it fell markedly, then rose for a time and either maintained this later level or fell toward the end, N. E. F., figure 5. The majority of cases therefore showed a period of low carbon dioxide tension just after the altitude was reached, followed by a more or less permanent rise. In two of the cases the low point was coincident with the arrival at 380 mm. This is interpreted to mean that the ventilation increases with the ascent and shows its maximum value shortly after the ascent is reached. Following this period there is a tendency toward more quiet breathing. This point will be discussed later in this paper when the data on the volume of breathing are presented.

The after-effects of exposures to a constant low oxygen level are shown in the sea level alveolar airs which were taken just after the subject reached 760 mm. again, that is, about 20 minutes after his exposure to 380 mm. In only four cases did it return to its previous average normal of 38.7 mm., the average normal after the experiment being 34.5 mm. This is shown particularly well in H. M. T. and B. R. L. in table 4. Both of these men responded by deep breathing and maintained an alveolar oxygen tension from 7 to 15 mm. higher than the average. The slow return of the carbon dioxide tension was pointed out by Boycott and Haldane (7), by Schneider (22), and by Douglas, Haldane, Henderson and Schneider (10). Schneider followed the carbon dioxide tension of H. H. R. who had lived on Pike's Peak for about six months. The level did not return to normal for nearly one month and a half and was accompanied by a change in the composition of the blood.

*The respiratory volume during reduction of barometric pressure.* Alveolar air determinations during the reduction of pressure at a rate equivalent to 1000 feet per minute indicate that an increase in ventilation takes place early and becomes most marked just after the subjects reach 428 or 380 mm. The volume per minute of breathing was there-

TABLE 5

*Experiments in the low pressure chamber with the Larsen spirometer. Subjects taken to 18,000 feet (380 mm.) in 18 minutes and held.*  
*Respiratory volume in liters*

| BAROMETER.....    | 760   |       |       |       | 656   |       |      |       | 560   |       |       |       |       | 480   |       |       |       | 410   |       | 395   |       | 385   |  |  |
|-------------------|-------|-------|-------|-------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|--|
|                   | 0     | 1     | 2     | 3     | 4     | 5     | 6    | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    | 16    | 17    | 18    | 19    | 20    | 21    |  |  |
| Minute .....      |       |       |       |       |       |       |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |  |  |
| E. C. S. 4/29/19  | 5.82  |       | 5.82  | 8.3   | 6.66  | 7.78  | 9.57 | 6.27  | 8.50  | 8.06  | 7.17  | 7.95  | 7.39  | 6.27  | 7.61  |       | 8.17  | 77.50 | 7.05  | 7.17  | 8.28  | 7.50  |  |  |
| K. O. N. 4/30/19  | 5.27  | 4.59  | 5.04  | 4.26  | 5.60  | 5.49  | 4.71 | 5.71  | 5.71  | 5.15  | 6.27  | 5.83  | 5.94  | 5.38  | 5.60  | 6.16  | 6.94  | 4.82  | 6.05  | 6.05  | 7.06  |       |  |  |
| N. E. B. 5/6/19   | 4.98  | 5.60  | 5.26  | 5.04  | 5.49  | 4.93  | 4.71 | 5.37  | 5.49  | 5.15  | 5.37  | 5.49  | 6.05  | 6.05  | 5.60  | 5.94  | 5.82  | 5.37  | 6.72  | 6.27  | 5.71  | 5.82  |  |  |
| B. R. L.* 5/29/19 | 7.06  | 7.39  | 6.16  | 6.04  | 7.39  | 8.28  | 8.96 | 7.95  | 8.96  | 10.33 | 9.07  | 9.40  | 9.85  | 9.63  | 11.20 | 10.60 | 12.30 | 11.90 | 13.00 | 13.30 | 12.70 | 13.90 |  |  |
| K. O. N.* 5/29/19 | 7.06  | 8.95  | 9.96  | 8.74  | 8.95  | 8.95  | 9.75 | 11.00 | 10.30 | 10.70 | 11.50 | 11.60 | 11.20 | 11.50 | 11.60 | 11.10 | 12.20 | 11.80 | 11.90 | 14.60 |       |       |  |  |
| E. C. S. 5/29/19  | 8.40  | 10.10 | 10.10 | 10.30 | 8.62  | 7.95  | 8.40 | 8.51  | 7.84  | 8.28  | 8.28  | 8.85  | 9.51  | 9.96  | 10.30 | 9.07  | 11.40 | 11.60 | 11.60 | 12.80 | 11.00 | 11.90 |  |  |
| G. M.* 5/29/19    | 9.07  | 9.07  | 10.40 | 8.17  | 7.50  | 9.74  | 8.51 | 6.84  | 8.74  | 8.85  | 8.74  | 8.96  | 8.51  | 10.50 | 11.90 | 9.52  | 10.10 | 9.74  | 12.20 | 18.40 |       |       |  |  |
| B. B. J.† 5/31/19 | 11.00 | 10.20 | 9.86  | 11.40 | 11.20 | 11.30 | 9.52 | 10.40 | 12.00 | 10.40 | 9.86  | 10.70 | 11.50 | 11.80 | 12.50 |       | 13.20 | 12.70 |       |       |       |       |  |  |
| B. R. L.† 6/2/19  | 8.74  | 7.61  | 7.17  | 6.27  | 7.72  | 5.71  | 7.72 | 7.95  | 9.85  | 9.07  | 10.30 | 8.51  | 9.74  | 10.60 | 11.00 | 11.90 | 14.00 | 13.10 | 15.70 | 14.10 |       |       |  |  |
| Average.....      | 7.49  | 7.94  | 7.75  | 7.61  | 7.68  | 7.79  | 7.98 | 7.78  | 8.60  | 8.44  | 8.51  | 8.59  | 8.85  | 9.07  | 9.70  | 9.18  | 10.50 | 9.84  | 10.50 | 11.60 |       |       |  |  |

\* Taken to 19,000 feet in 19 minutes and held.

† Taken to 17,000 feet in 17 minutes and held.

fore investigated. The per-minute volume of breathing was measured with a Larsen spirometer in men reduced to pressures of 395 mm., 380 mm., 365 mm. (17,000, 18,000 and 19,000 feet respectively). The data are shown in table 5. The subjects all showed a considerable increase in breathing varying between 1.8 and 9.3 liters, or 34 and 103 per cent. The average amount breathed per minute at 760 mm. was 7.49 liters. Just as soon as the reduction started the average figure went to 7.94 liters, due no doubt to anxiety of some of the subjects. By the third minute it had fallen to 7.61 liters. The readings thereafter showed a progressive increase until at the 19th minute the average figure was 11.59 liters, an increase of 54.7 per cent. It will be seen both from the average figures and from the individual cases that the onset of increased breathing started usually between the fourth and sixth minutes or between 656 and 605 mm., that is, 4000 and 6000 feet. This confirms the alveolar air findings reported above. The onset of increased breathing due to low oxygen produced by the rebreathing method was reported by Schneider (3) to begin in some cases as early as 16 per cent oxygen, corresponding to about 7000 feet or 580 mm., and by Ellis (23) before 17.5 per cent oxygen was reached. Schneider (3) found in the rebreathing test that the rate remained unchanged for many men but the majority increased the rate by 2 to 4 breaths per minute. The depth of breathing he found increased from 20 to 128 per cent when at 8.5 to 6 per cent oxygen.

*The respiratory volume during maintained low barometric pressure.* In the majority of alveolar air determinations the lowest carbon dioxide figure was found shortly after the reduced barometric pressure was attained. Thereafter the carbon dioxide level either rose slowly or was maintained. Experiments in which the volume per minute of breathing was measured during a reduction of pressure to 380 mm. at the usual rate and during the following 48 to 84 minutes of the maintained low barometric pressure are tabulated in table 6. Control experiments at 760 mm. using the mask and meter are also shown. The figures given are the three-minute averages in liters. All of the eleven subjects showed an increase in lung ventilation usually most marked within 10 minutes after 380 mm. was reached. Seven showed a reduction of ventilation thereafter continuing until the end of the experiment. Two showed a reduction followed by a terminal rise, which in the case of A. F. H. was very marked. Two cases showed a very slow rise which tended to be maintained until the end. The usual type of response is shown in figure 6, in which the cases of I. M., W. H. G.,

P. S. B. and E. A. R. are plotted. In three subjects taken to 380 mm. at the same rate and held from 59 to 81 minutes the Larsen spirometer was used. Two showed this type of response and in one the increase in respiration continued until the end.

The typical response seen in nine cases out of fourteen in which the volume of breathing was measured, corresponds to that observed in six of the nine cases in which the alveolar air tensions were followed under similar conditions. In one case the subject was taken to 12.5 per cent oxygen in 17 minutes by the rebreathing method and held at



Fig. 6. The respiratory per-minute volume of four cases in liters, taken to 380 mm. (18,000 feet) in 18 minutes and maintained at that level. Note the decrease in ventilation after the maximum is reached. This corresponds to the alveolar  $\text{CO}_2$  tensions under similar conditions shown in figure 5.

that level for 68 minutes. The response was similar to the typical low pressure chamber experiment. His per-minute ventilation increased from 8.3 liters to 10.4 liters at the 14th minute. It held a level at about 9.2 liters from that time until the 40th minute, when it gradually fell to 8.4 liters at the 84th minute.

Several subjects taken to 10 per cent oxygen in from 17 to 20 minutes by the Dreyer method showed similar responses. In these cases the ventilation was indicated by a Fitz pneumograph and the average amplitude times the rate per minute was taken as a figure to indicate the per-minute ventilation. I. M. increased from 90 to 252 at the 17th



TABLE 6

*Experiments with gas mask and meter. Subject sitting. Respiration in liters per minute. Controls at 760 mm.*

| MINUTE.....           | 0    | 3   | 6   | 9   | 12  | 15  | 18  | 21  | 24  | 27  | 30  | 33  | 36  | 39  | 42  | 45  | 48  | 51  | 54  | 57  | 60  | 63  | 66  | 69  | 72  | 75  | 78  | 81  | 84  | 87  | 90  |  |
|-----------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| C. R. S. 6/8/18.....  | 5.85 | 2.4 | 6.5 | 0.5 | 0.1 | 2.5 | 3.1 | 8.4 | 6.1 | 8.1 | 3.4 | 9.1 | 4.1 | 4.8 | 1.7 | 4.2 | 1.4 | 4.5 | 0.4 | 4.7 |     |     |     |     |     |     |     |     |     |     |     |  |
| W. H. G. 7/10/18..... | 6.37 | 6.8 | 2.6 | 9.5 | 7.6 | 9.6 | 3.6 | 4.6 | 2.6 | 7.6 | 4.7 | 5.6 | 6.4 | 6.9 | 5.7 | 7.5 | 7.0 | 6.6 | 6.9 |     |     |     |     |     |     |     |     |     |     |     |     |  |
| J. J. G. 7/11/18..... | 9.29 | 9.9 | 2.9 | 2.9 | 3.9 | 0.7 | 9.0 | 8.8 | 7.7 | 9.0 | 8.4 | 9.7 | 8.4 | 8.5 | 9.1 | 8.7 | 8.8 | 8.3 | 8.7 | 8.3 | 8.5 | 8.1 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 | 8.7 | 8.7 |  |

*Experiments in the low pressure chamber. Subjects taken to 18,000 feet (380 mm.) in 18 minutes and held*

|          |              |      |     |     |     |     |     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |      |      |      |      |     |     |     |     |     |     |
|----------|--------------|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|------|------|------|------|------|-----|-----|-----|-----|-----|-----|
| L. M.    | 7/1/18.....  | 5.54 | 4.5 | 1.5 | 4.5 | 8.6 | 4.7 | 1.7  | 4.6 | 9.7 | 3.7 | 9.7 | 7.1 | 7.5 | 6.9 | 6.9  | 6.7 | 5.4 | 6.6 | 3.6 | 7.4  | 5.7  | 6.3  | 6.7  | 6.7  | 5.9  | 6.5 | 5.7 |     |     |     |     |
| E. W. B. | 6/30/18..... | 8.19 | 5.8 | 7.8 | 3.8 | 3.8 | 6.9 | 2.1  | 0.8 | 0.9 | 3.8 | 6.9 | 2.7 | 9.1 | 8.4 | 9.0  | 8.1 | 6.7 | 7.8 | 9.8 | 9.8  | 8.1  | 8.6  | 9.3  | 8.7  | 9.0  | 9.2 | 8.9 | 9.6 | 9.1 | 7.9 | 7.7 |
| B. F.    | 7/2/18.....  | 8.58 | 2.7 | 6.7 | 9.8 | 1.8 | 6.8 | 9.9  | 8.9 | 4.8 | 9.9 | 1.8 | 9.8 | 8.7 | 9.2 | 8.1  | 7.8 | 8.9 | 4.8 | 5.8 | 8.1  | 8.3  | 8.2  |      |      |      |     |     |     |     |     |     |
| D. T. R. | 7/3/18.....  | 7.27 | 7.8 | 3.7 | 3.7 | 8.7 | 8.7 | 9.3  | 7.6 | 8.1 | 7.7 | 8.4 | 4.7 | 4.7 | 7.8 | 6.9  | 7.9 | 4.7 | 3.6 | 8.6 | 9.7  | 7.4  | 7.3  | 7.3  | 7.6  | 7.3  | 8.0 | 7.1 | 7.0 | 7.5 | 7.3 |     |
| H. J. M. | 7/5/18.....  | 5.45 | 7.5 | 9.4 | 6.5 | 9.5 | 7.5 | 6.6  | 7.6 | 0.6 | 2.6 | 1.6 | 6.4 | 5.6 | 6.9 | 6.5  | 6.5 | 8.5 | 9.5 | 0.5 | 8.6  | 6.4  | 6.6  | 6.6  |      |      |     |     |     |     |     |     |
| A. F. H. | 7/8/18.....  | 6.76 | 8.5 | 4.4 | 9.6 | 2.8 | 4.9 | 1.9  | 4.9 | 0.9 | 1.0 | 2.9 | 2.8 | 9.4 | 8.3 | 8.8  | 2.7 | 9.7 | 2.2 | 7.0 | 7.5  | 8.7  | 7.5  | 8.7  | 8.7  | 7.3  | 7.1 | 6.3 | 5.8 | 4.9 | 6.0 |     |
| P. S. B. | 7/9/18.....  | 6.76 | 8.7 | 1.6 | 9.7 | 2.7 | 5.8 | 1.9  | 0.8 | 3.8 | 1.8 | 1.8 | 8.4 | 8.6 | 8.0 | 8.0  | 8.3 | 8.3 | 8.2 | 9.8 | 8.6  | 8.5  | 8.5  | 8.5  | 8.5  | 8.5  | 8.5 | 8.5 | 8.5 | 8.5 | 8.5 |     |
| W. H. G. | 7/10/18..... | 5.76 | 6.6 | 3.7 | 1.6 | 6.6 | 9.7 | 0.8  | 1.6 | 5.7 | 8.7 | 2.2 | 7.6 | 7.0 | 7.6 | 6.6  | 8.6 | 8.7 | 2.6 | 8.6 | 6.5  | 7.0  | 6.6  | 6.6  | 6.6  | 6.6  | 6.6 | 6.6 | 6.6 | 6.6 | 6.6 |     |
| A. F. H. | 7/15/18..... | 7.87 | 6.7 | 9.7 | 8.7 | 4.7 | 7.8 | 2.9  | 7.9 | 3.8 | 7.9 | 2.2 | 8.9 | 9.5 | 9.0 | 10.2 | 8.7 | 6.8 | 3.8 | 7.9 | 11.4 | 13.8 | 15.5 | 16.5 | 19.8 | 19.1 |     |     |     |     |     |     |
| E. A. R. | 7/13/18..... | 7.87 | 6.7 | 5.8 | 8.3 | 9.8 | 8.8 | 10.1 | 8.2 | 9.6 | 8.8 | 8.9 | 9.8 | 8.0 | 9.1 | 8.7  | 9.7 | 8.8 | 8.2 | 8.1 | 8.7  | 8.7  | 8.7  | 8.7  | 8.7  | 8.7  | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 |     |
| W. C. W. | 7/16/18..... | 6.66 | 2.6 | 1.6 | 1.6 | 9.5 | 9.6 | 0.7  | 0.6 | 5.6 | 6.6 | 6.9 | 6.9 | 7.6 | 7.6 | 7.0  | 6.7 | 6.5 | 7.4 | 6.1 | 6.4  | 6.7  | 6.7  | 6.7  | 6.7  | 6.7  | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |     |

minute and then fell to 154 at the 90th minute. This is a picture similar to that of I. M. in table 6 and figure 6. C. L. S. in a similar experiment went from 192 to 360 at the 17th minute and then fell to 128 at the 95th minute.

The rate of breathing was reported in seven cases during the reduction of pressure and the holding period in the low pressure chamber. In four cases it fell from two to five breaths per minute as the experiment proceeded. The rates of W. H. G. and P. S. B. are plotted in figure 6. One showed no change in rate. Two showed an increase, one, N. E. B., from 13 per minute to 15 at 380 mm. and then to 19 at the 82nd minute when the low pressure was maintained. The other, A. F. H., showed no increase until the 58th minute when the rate per minute started to rise from 17 to 38 at the 77th minute. The per-minute volume of breathing increased markedly, as will be seen in table 6.

The tidal air has never been observed to decrease in the low pressure chamber. The majority of subjects responded to the low oxygen exposures by deep slow breathing although frequently Cheyne-Stokes breathing has been observed.

#### DISCUSSION

The relation of respiration to low oxygen tension presented in this paper is, in a general way, in accord with most of the literature. The early response to decreased oxygen tension and the tendency of the breathing to return toward the normal during maintained low oxygen which we find under the conditions of our experiments, may appear at first sight to be contrary to the views which have been presented by Haldane and others.

Haldane and Smith (24) in 1893 found marked hyperpnoea when the oxygen was reduced to 12 per cent, the carbon dioxide being removed. They write

the fact that any hyperpnoea should have been caused by a reduction of oxygen to 12 per cent may seem at first sight to be hardly consistent with our former conclusions that hyperpnoea caused by vitiated air is entirely due to carbon dioxide.

They explain that in the former carbon dioxide and low oxygen experiments the increased supply of oxygen brought about by the carbon dioxide hyperpnoea prevented an extra hyperpnoea due to want of oxygen from developing. Haldane and Poulton (2) in 1908 reported experiments in which the subjects reduced 25 liters of air from 9

or 10 per cent oxygen to 4 or 5 per cent in less than 10 minutes. They found marked hyperpnoea which they believed was not due to the direct effect of oxygen want but to lowering of the threshold of the respiratory center to carbon dioxide which has not had time to escape. In their experiments, however, the alveolar carbon dioxide fell to between 3.2 and 4 per cent. In another group of experiments the oxygen per cent in the inspired air was reduced to about 9 per cent in from 15 to 23 minutes. In these "no noticeable hyperpnoea" is reported, although the alveolar carbon dioxide fell to between 3.9 and 4.3 per cent, which indicates that a considerable increase in ventilation must have occurred. Haldane, Meakins and Priestley (25) in 1919 conclude, from exposures to low oxygen of about 10 per cent, lasting about 6 minutes, that the first result of diminution in the percentage of oxygen is an increase in the depth of respiration owing to a lowering of the threshold-exciting value of carbon dioxide. This is followed by a period of periodic breathing due to the much quicker action of want of oxygen as compared with that of increase of carbon dioxide. Further reduction of the oxygen percentage showed the periodicity replaced by a very rapid shallow breathing. They write, "Want of oxygen in the inspired air causes shallow breathing which in turn intensifies the anoxemia." The point of view taken by these authors is that after the first period during which the threshold is lowered to carbon dioxide, oxygen want acts as a paralyzing agent on the respiratory center.

We believe that one is not justified in drawing too general conclusions regarding the effects of oxygen want from experiments of extreme degree and short duration. We shall show in a later paper the quick respiratory and circulatory responses to the breathing of pure nitrogen. At the other extreme is the well-known ascent of the Duke of the Abruzzi in the Himalayas to 24,580 feet. In quick extreme anoxemia, respiratory and circulatory factors respond quickly and to their greatest capacity. If the exposure to low oxygen is slow and long-continued, other factors have time to assist in the compensation. We feel, therefore, that the rate of exposure as well as the degree is an important condition when considering the effects of oxygen want. The lack of recognition of this fact brings about confusion. We have never seen a case of shallow breathing and only two cases of increased rate under the conditions of our experiments. They are, however, quite different from those of Haldane, Meakins and Priestley. The decrease in the respiratory per-minute volume, which occurs in our experiments after

the preliminary increase with the reduction of pressure, takes place during exposures of from 30 to 120 minutes rather than during exposures of from 6 to 10 minutes. We do not believe it to be a sign of failing respiratory center, but an indication of improvement in condition, as will be pointed out in another paper.

#### SUMMARY

1. Twenty-four men were taken to 352 mm. pressure in a low pressure chamber at a rate equivalent to an ascent of 1000 feet per minute. In these cases the average alveolar oxygen tension fell 66 per cent, and the alveolar carbon dioxide fell 24 per cent.

2. The average carbon dioxide tension was definitely lowered at 656 mm. (4000 feet) which indicates that the onset of increased breathing had occurred.

3. Alveolar tensions taken during a reduction of pressure to 380 mm. (18,000 feet) at the usual rate, and during the subsequent 30 to 120 minutes while the low pressure level was maintained, showed that after the preliminary fall in carbon dioxide tension there was a tendency for this tension to rise for a time although it remained low during the holding period. After 760 mm. had been reached again within 20 minutes, the carbon dioxide had not recovered its former level in the majority of cases.

4. The lowest carbon dioxide tension occurred about 5 minutes after 380 mm. was reached, when the reduction was equivalent to an ascent of 1000 feet per minute. In some cases this latent period did not occur and the maximum breathing was coincident with the arrival at 380 mm.

5. Tensions taken while the pressure was maintained at 428 mm. (15,000 feet) did not show the same profound effects. The carbon dioxide tension did not fall so far and maintained a level.

6. Both oxygen and carbon dioxide alveolar tensions responded quickly to rapid successive reductions of barometric pressure to 428 mm.

7. The per-minute volume of breathing was determined for each minute during a reduction of pressure at the usual rate to 395, 380 and 365 mm. The majority of cases showed a definite increase in ventilation taking place between 656 and 605 mm. (4000 and 6000 feet). This final increase amounted to an average of 54.7 per cent. Individual cases varied from 34 to 103 per cent increase.

8. The per-minute volume of breathing was determined during a reduction of pressure to 380 mm. at the usual rate, and during a period



of from 48 to 84 minutes while the low level was maintained. In nine out of fourteen cases the maximum ventilation occurred within 10 minutes after 380 mm. was reached. Following this period there was a distinct falling off in the per-minute volume. These cases correspond to the six cases out of nine in which the alveolar carbon dioxide showed a rise after the preliminary fall.

9. The decrease in the per-minute volume of breathing after the first maximum value, as 380 mm. was reached, was also found in cases in which the low oxygen tension was produced by the rebreathing method and by the Dreyer nitrogen dilution method.

10. The partial return of the respiration toward the normal is believed to indicate a temporary improvement in condition.

11. Alveolar air tensions taken during a reduction of the oxygen partial pressure by the rebreathing method or the nitrogen dilution method corresponded to those taken under reduced barometric pressure.

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## COMPENSATORY REACTIONS TO LOW OXYGEN

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In earlier papers we have dealt separately with the blood, circulatory and respiratory changes induced by short periods of exposure to lowered oxygen tensions. It was shown that men responded with definite adaptive physiological changes when subjected to gradually decreasing oxygen partial pressures which reached values between 76 and 51 mm. Hg., corresponding to barometric pressures of from 360 to 240 mm. (19,200 to 29,000 feet), and also when kept for from 30 to 130 minutes at oxygen partial pressures of from 88 to 80 mm., corresponding to barometric pressures of from 425 to 380 mm. (15,000 to 18,000 feet). In approximately 78 per cent of all men examined the erythrocytes and hemoglobin increased in a unit volume of blood. This increase did not occur immediately but usually required between 40 and 60 minutes to become definite. About 13 per cent of all cases showed a well-defined increase in hemoglobin within 26 minutes (1).

The heart responded to slight changes in oxygen tension by an acceleration in the rate of beat. Some men gave the first response at an oxygen partial pressure of 137 mm., barometric pressure 656 mm. (4000 feet); but in the majority the acceleration began between oxygen partial pressures of from 113 to 128 mm., barometric pressures of from 610 to 542 mm. (6000 to 8800 feet). Evidence of an increased rate of blood flow was found in the acceleration of the heart rate, and in a fall in the diastolic blood pressure which resulted in an augmented pulse pressure. When a constant level of oxygen was maintained, the heart reached the maximum rate after a lapse of a period of variable length. It continued at the maximum rate for some time after which the rate retarded somewhat. The evidence indicated that a marked and progressive increase in the rate of blood flow occurred during the reduction and early holding period, after which there followed a period of more or less constant rate of flow. Later in many subjects, as shown by the heart retardation and the rise in the diastolic pressure, the flow of blood in some degree approached the normal rate (2).

The per-minute volume of breathing showed a definite increase between 656 and 605 mm. (4000 and 6000 feet). In the majority of cases the maximum ventilation occurred within 10 minutes after 380 mm. was reached. Following this period there was a distinct falling off in the per-minute volume (3).

In the present paper we propose to consider the relative values of the compensatory reactions to low oxygen tensions. Men differ in sensitiveness to lowered oxygen and in the power to make physiological adaptations which will, from a decreased supply, provide sufficient oxygen to maintain tissue and body efficiency. In some there is an immediate or at least an early response to a decrease in oxygen, in others the response occurs much later and may be less adequate. Some men make excellent compensations to low oxygen tensions while others show insufficient compensations at only moderately low oxygen. Individuals differ also in the use of the several ways of responding to the decrease in oxygen. The majority of men appear to make a well-balanced use of the three mechanisms for supplying oxygen. The ventilation of the lungs, the rate of blood flow and the percentage of red corpuscles and hemoglobin are definitely increased. Some meet the new condition largely by increased respiration and others depend almost entirely upon an increased blood flow. In many individuals, during the early period of exposure to a decreasing oxygen, the burden of compensation is borne wholly by the circulatory and respiratory mechanisms, but later the blood changes relieve one or both of these mechanisms from a part of the burden. Our data show an interdependence and an interplay of the adaptive mechanisms when a subject is held under a constant low oxygen tension. Schneider (4) has reported briefly several cases in which the interplay was present.

The majority of the experiments which have been presented in part in our earlier papers were conducted in the low pressure chamber. The barometric pressure was lowered to 425, 395 or 380 mm. (15,000, 17,000 or 18,000 feet) at the rate of 1000 feet per minute, and held at that pressure for periods varying from 30 to 130 minutes. In a smaller number of experiments the subject breathed atmospheric air diluted with nitrogen by the Dreyer method. Starting with undiluted air, 20.96 per cent oxygen, the nitrogen was added gradually in greater and greater proportion, so that at the end of 20 minutes the mixture contained only 10 per cent oxygen. This percentage of oxygen was then maintained for from 30 to 90 minutes. Thus the subject was kept under low oxygen for a period of from 50 to 112 minutes.

In the low pressure experiments the observers were given oxygen by means of a tube held in the mouth. It was, therefore, necessary to determine whether oxygen accumulated within the chamber during the period of experimentation. In the majority of experiments samples of air were taken 3 to 5 times during the experiment, and later analyzed for oxygen and carbon dioxide. The exhaustion pump was kept working continuously throughout an experiment so that sufficient ventilation was maintained to prevent an accumulation of carbon dioxide. Often there was some accumulation of oxygen, but it was found to reach quickly a constant level. With such data a corresponding correction for altitude was sometimes made. We have, however, many experiments in which no accumulation occurred, and we have usually omitted the correction when the accumulation was slight and the oxygen percentage remained constant during the holding period. We are satisfied that the interpretation of our data is not vitiated by this accumulation. As shown in our earlier papers, the effects upon the blood, circulation and respiration were the same under the three methods used for providing low oxygen tensions. Since this was found to be the case, we have demanded only a constant oxygen tension during the holding period.

#### THE LOW PRESSURE CHAMBER EXPERIMENTS

The interplay of the three adaptive responses has been studied in forty-seven experiments. For convenience of discussion we have divided the reactions observed during the period at which the barometric pressure remained constant into four groups: *a*, Cases in which the pulse retarded after maintaining a high rate for a period of variable length, and the hemoglobin percentage of the blood increased; *b*, cases in which the pulse maintained the new level after an increase in rate, and the percentage of hemoglobin increased; *c*, cases in which the pulse rate remained constant and the hemoglobin did not increase; *d*, a few cases in which the pulse rate retarded and the hemoglobin did not increase. The variations in respiration have been determined for each of the groups.

*a. Retardation of the pulse rate during the holding period with an increase in hemoglobin.* There were twenty-six cases in which an increase of hemoglobin seemed to favor the heart and sometimes the respiration. The beneficial cardiac effect, as we interpret the data, was manifested by a slowing of the pulse rate and frequently by a decrease in the blood



flow shown by a rise in the diastolic pressure and a corresponding decrease in the pulse pressure. No two cases were exactly the same. The interdependence of the three compensatory responses can best be shown by a detailed study of a few individual cases.

*N. E. F. June 7, 1918. Barometric pressure 380 mm.*

|                                | MINUTE |     |      |      |      |      |      |      |     |
|--------------------------------|--------|-----|------|------|------|------|------|------|-----|
|                                | 0      | 5   | 10   | 15   | 25   | 35   | 55   | 75   | 85  |
| Pulse.....                     | 73     | 75  | 75   | 79   | 82   | 79   | 76   | 72   |     |
| Systolic.....                  | 114    | 112 |      |      | 100  | 102  | 104  | 100  |     |
| Diastolic.....                 | 70     | 72  |      |      | 56   | 58   | 58   | 54   |     |
| Pulse pressure.....            | 44     | 40  |      |      | 44   | 44   | 46   | 46   |     |
| Alveolar O <sub>2</sub> .....  | 106    |     | 65.2 | 41.5 | 37.4 | 37.0 | 33.6 | 32.3 |     |
| Alveolar CO <sub>2</sub> ..... | 39.1   |     | 35.8 | 30.0 | 28.7 | 32.0 | 34.4 | 33.8 |     |
| Hemoglobin.....                | 94     |     |      |      | 96   | 96   |      | 97   | 100 |

This subject was in good condition up to the 85th minute when blood was drawn from a vein. It will be observed that during the period of ascent the pulse rate accelerated and the alveolar carbon dioxide tension fell. Thus the burden of compensation to decreasing oxygen was at first borne by the circulation and respiration. The pulse rate reached its maximum four minutes after the barometric pressure of 380 mm. was attained. About this time, the 25th minute, the blood flow as judged from the pulse rate and pulse pressure reached its maximum. Coincident with this the per-minute ventilation of the lungs was greatest as indicated by the carbon dioxide which at this time was only 28.7 mm. The circulatory conditions remained about the same during the next 10 minutes, but the breathing, as judged from the carbon dioxide, was lessened. Both the circulation and the ventilation of the lungs fell off from this time up to about the 60th minute, after which they remained constant to the end of the experiment. It should be noted that the hemoglobin had begun to increase at the 25th minute and continued until the close of the experiment. Coincident with this increase in hemoglobin there was a retardation in the pulse rate and a decrease in the breathing. The interplay of compensatory factors for this case is shown graphically in figure 1.

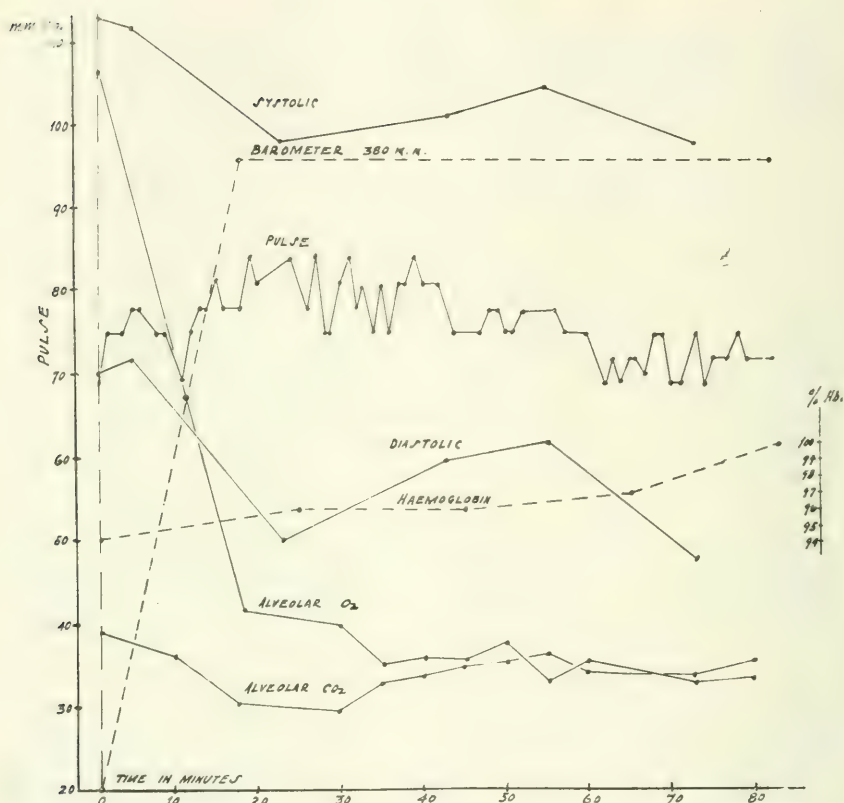


Fig. 1. N. E. F., June 7, 1918. Taken to 380 mm. (18,000 feet) in 18 minutes in the low pressure chamber and maintained at that level. This case illustrates the inter-relation of pulse rate, respiration and oxygen-carrying-capacity of the blood.

R. S. S. June 6, 1918. Barometric pressure 380 mm.

|                                | MINUTE |    |    |     |      |      |      |      |
|--------------------------------|--------|----|----|-----|------|------|------|------|
|                                | 0      | 5  | 10 | 15  | 25   | 35   | 45   | 75   |
| Pulse.....                     | 70     | 74 | 76 | 80  | 94   | 90   | 95   | 88   |
| Systolic.....                  | 118    |    |    | 114 | 114  | 110  | 106  | 100  |
| Diastolic.....                 | 70     |    |    | 64  | 54   | 48   | 44   | 42   |
| Pulse pressure.....            | 48     |    |    | 50  | 60   | 62   | 62   | 58   |
| Alveolar O <sub>2</sub> .....  | 97.8   |    |    |     | 32.7 | 33.2 | 32.1 | 32.2 |
| Alveolar CO <sub>2</sub> ..... | 38.8   |    |    |     | 32.7 | 33.5 | 32.4 | 34.2 |
| Hemoglobin.....                | 102    |    |    |     | 104  | 104  | 106  | 106  |

In this subject there was a progressive increase in the rate of blood flow, as shown by the pulse rate and pulse pressure, which reached the maximum at the 45th minute. The pulse rate reached its maximum 10 minutes earlier. We believe that this illustrates that the pulse rate alone did not determine the maximum compensation in circulation. The fall in diastolic pressure with the resulting increase in pulse pressure is considered evidence of vasodilatation in the systemic circulation. Judging by the decrease in pulse rate and in pulse pressure, the rate of blood flow began to slow at about the 58th minute. The respiration attained its maximum soon after a pressure of 380 mm. was reached, and then maintained a fairly constant per-minute volume of ventilation until the end of the experiment. The hemoglobin showed a slight increase at the 25th minute and reached its maximum concentration at about the 50th minute. In this experiment the circulation seems to have been favored by the concentration in hemoglobin while the increase in respiration was maintained throughout.

*B. M. L. June 11, 1918. Barometric pressure 380 mm.*

|                                | MINUTE |    |    |      |      |          |      |      |
|--------------------------------|--------|----|----|------|------|----------|------|------|
|                                | 0      | 5  | 10 | 15   | 25   | 35       | 55   | 75   |
| Pulse.....                     | 68     | 69 | 70 | 78   | 89   | 91       | 87   | 85   |
| Systolic.....                  | 102    |    |    |      | 102  | 102      | 102  | 102  |
| Diastolic.....                 | 68     |    |    |      | 52   | 48       | 46   | 46   |
| Pulse pressure.....            | 34     |    |    |      | 50   | 54       | 56   | 56   |
| Alveolar O <sub>2</sub> .....  | 109    |    |    | 35.8 | 32.0 | 31.8     | 31.9 | 32.4 |
| Alveolar CO <sub>2</sub> ..... | 37     |    |    | 36.6 | 32.9 | 33.0     | 31.1 | 30.3 |
| Hemoglobin.....                | 96     |    |    |      | 96   | 40th, 99 | 106  | 104  |

In this case the systolic pressure remained constant throughout while in the cases of N. E. F. and R. S. S. it fell. The increase in pulse pressure, as in the case of R. S. S., is again definite and is determined wholly by a fall in the diastolic pressure. The rate of blood flow reached its maximum at about the 35th minute, which was approximately the time of maximum pulse rate. From this time the pulse rate fell gradually about 9 per cent, while the pulse pressure remained high and increased slightly, with the result that the rate of blood flow was presumably reduced. The respiration increased early and then maintained a level until the 50th minute, after which it increased gradually until the end of the experiment. The hemoglobin did not begin to increase until between the 26th and 40th minutes. The pulse rate increased

until the 32nd minute. This suggests a relationship between the hemoglobin and the pulse rate. The increase in breathing is also a factor that may have permitted a slowing of the heart rate.

*K. O. N. April 30, 1919. Barometric pressure 380 mm.*

|                     | MINUTE |    |    |    |     |     |     |     |
|---------------------|--------|----|----|----|-----|-----|-----|-----|
|                     | 0      | 5  | 10 | 15 | 25  | 35  | 55  | 75  |
| Pulse.....          | 84     | 84 | 89 | 94 | 102 | 98  | 95  | 92  |
| Systolic.....       | 116    |    |    |    | 122 | 120 | 116 | 116 |
| Diastolic.....      | 68     |    |    |    | 64  | 58  | 56  | 54  |
| Pulse pressure..... | 48     |    |    |    | 58  | 62  | 60  | 62  |

|                      | MINUTE |     |           |     |          |     |           |     |     |     |     |  |
|----------------------|--------|-----|-----------|-----|----------|-----|-----------|-----|-----|-----|-----|--|
|                      | 0      | 10  | 15        | 18  | 20       | 32  | 36        | 42  | 49  | 59  | 74  |  |
| Respiration volume.. | 5.3    | 5.5 | 5.7       | 5.9 | 6.6      | 5.7 | 6.0       | 5.2 | 5.4 | 5.5 | 5.6 |  |
| Hemoglobin.....      | 100    |     | 27th, 101 |     | 52d, 106 |     | 74th, 106 |     |     |     |     |  |

In this case we determined the per-minute volume of breathing in liters and took the alveolar air occasionally to compare with the volume. The blood flow and respiration each reached the maximum at once on arriving at 380 mm. The pulse rate then held until the 35th minute when it fell slowly until the fall was 12 per cent at the end. The respiratory volume fell slowly until the 42nd minute, after which it held at a volume slightly above the normal ventilation. The hemoglobin was just beginning to concentrate at the 27th minute. It reached its maximum by the 52nd minute. In this case the circulatory and respiratory mechanisms seemed to have been relieved somewhat by the increase in hemoglobin.

In figure 2 the data for P. S. B., July 7, 1918, has been plotted. The pulse rate and diastolic pressure changes indicate that the blood flow reached its maximum during the early part of the holding period, and also that toward the end it decreased markedly. The decrease occurred when the hemoglobin had increased. The respiration was not benefited by the increase in hemoglobin. The remaining twenty-one cases in this group show in a similar manner the relationship between the increase in hemoglobin and the retardation in the pulse rate. We believe that this group of cases represents the usual reaction when the transition from normal oxygen tension to low oxygen is made gradually and at a moderately rapid rate.



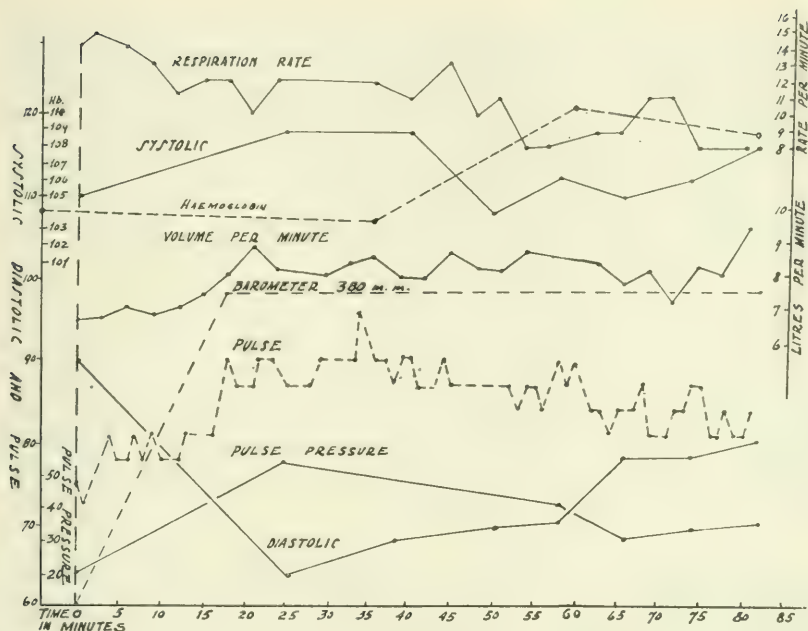


Fig. 2. P. S. B., July 9, 1918. Taken to 380 mm. (18,000 feet) in 18 minutes in the low pressure chamber. This case illustrates the interplay of compensatory factors, particularly the blood flow and the oxygen carrying capacity.

*b. Maximum pulse rate maintained with an increase in hemoglobin.* There were nine cases in this group, four of which are presented here.

G. C. W. June 25, 1918. Barometric pressure 380 mm.

|                                | MINUTE |          |    |      |      |      |      |     |
|--------------------------------|--------|----------|----|------|------|------|------|-----|
|                                | 0      | 5        | 10 | 15   | 25   | 35   | 55   | 75  |
| Pulse.....                     | 60     | 62       | 64 | 70   | 78   | 78   | 78   | 82  |
| Systolic.....                  | 100    |          |    |      | 120  | 120  | 118  | 114 |
| Diastolic.....                 | 60     |          |    |      | 64   | 58   | 48   | 48  |
| Pulse pressure.....            | 40     |          |    |      | 56   | 62   | 70   | 66  |
| Alveolar O <sub>2</sub> .....  | 98.5   |          |    | 36.2 | 29.7 | 30.4 | 31.8 |     |
| Alveolar CO <sub>2</sub> ..... | 37.1   |          |    | 29.6 | 32.7 | 32.3 | 23.1 |     |
| Hemoglobin.....                | 98     | 82d, 100 |    |      |      |      |      |     |

In this case first the pulse, then the systolic pressure, and then the diastolic pressure each in turn aided in maintaining an increased rate of blood flow. The subject appeared to be compensating satisfactorily but his reactions seemed to be insufficient in that the circulatory and respiratory reactions continued to increase even toward the end of the

experiment. The pulse rate was higher after the 70th minute than at any time before. The respiration increased gradually but not so much as in the average case, until the 45th minute, when a marked increase in ventilation took place lowering the carbon dioxide from 31.2 mm. to 22.4 mm. The fact that the respiration increased markedly without affecting the pulse rate shows that the demand for oxygen was not sufficiently cared for. The increase in hemoglobin was slight and not in evidence until the end.

*W. C. W. July 16, 1918. Barometric pressure 380 mm.*

|                     | MINUTE |    |     |     |     |     |     |     |
|---------------------|--------|----|-----|-----|-----|-----|-----|-----|
|                     | 0      | 5  | 10  | 15  | 25  | 35  | 55  | 75  |
| Pulse.....          | 66     | 69 | 72  | 75  | 88  | 86  | 82  | 90  |
| Systolic.....       | 104    |    | 106 | 104 | 102 | 104 | 104 | 102 |
| Diastolic.....      | 70     |    |     |     | 52  | 44  | 64  | 60  |
| Pulse pressure..... | 34     |    |     |     | 50  | 60  | 40  | 42  |

|                         | MINUTE |           |     |     |           |     |     |     |     |     |
|-------------------------|--------|-----------|-----|-----|-----------|-----|-----|-----|-----|-----|
|                         | 0      | 5         | 10  | 27  | 30        | 36  | 39  | 54  | 75  | 84  |
| Respiration volume..... | 6.6    | 6.2       | 6.2 | 6.9 | 6.9       | 7.6 | 7.0 | 7.4 | 6.5 | 7.1 |
| Hemoglobin.....         | 98     | 64th, 102 |     |     | 78th, 104 |     |     |     |     |     |

The heart rate at the 20th minute was 90. It then varied markedly for the next 40 minutes but at the 65th minute it reached 90 once more and showed a tendency to go higher, reaching 95 at times. The respiration reached its maximum per-minute volume at about the 36th minute and maintained it until the 54th minute, after which it decreased slightly. The hemoglobin had increased definitely by the 64th minute. In this experiment, if the hemoglobin exerted any sparing action it was shown in respiration. Compensation seemed to be somewhat inadequate.

*E. C. S. April 24, 1919. Barometric pressure 380 mm.*

|                     | MINUTE |    |    |    |    |     |     |     |     |
|---------------------|--------|----|----|----|----|-----|-----|-----|-----|
|                     | 0      | 5  | 10 | 15 | 25 | 35  | 55  | 75  | 95  |
| Pulse.....          | 76     | 76 | 80 | 84 | 98 | 100 | 108 | 105 | 102 |
| Systolic.....       | 108    |    |    |    |    | 128 | 134 |     | 126 |
| Diastolic.....      | 62     |    |    |    |    | 62  | 58  |     | 58  |
| Pulse pressure..... | 46     |    |    |    |    | 66  | 76  |     | 68  |

|                         | MINUTE |           |     |           |     |           |     |     |     |
|-------------------------|--------|-----------|-----|-----------|-----|-----------|-----|-----|-----|
|                         | 0      | 17        | 25  | 33        | 41  | 56        | 81  | 88  | 99  |
| Respiration volume..... | 5.8    | 7.4       | 8.5 | 8.5       | 7.4 | 7.6       | 7.2 | 6.6 | 6.6 |
| Hemoglobin.....         | 100    | 29th, 108 |     | 57th, 107 |     | 77th, 109 |     |     |     |

The rate of blood flow undoubtedly increased markedly up to the 35th minute. The pulse rate rose early to a first high point (100), then held on a plateau until the 39th minute, after which it again accelerated until the 46th minute, when it held more or less constant until the end. A slight lowering appeared at the 95th minute. The respiration increased during the ascent and attained its maximum at the 23rd minute. It held this level until the 36th minute, after which the per-minute volume fell and maintained a new level until the 88th minute. During the interval from the 36th to the 42nd minutes, while the respiration was being reduced, the pulse rate rose to its second high point (105). The hemoglobin showed concentration at the 29th minute. In this experiment the respiration seems to have been spared by the increase in hemoglobin. An interplay between circulation and respiration was present during the middle period, from the 36th to the 46th minutes.

*N. E. B. May 6, 1919. Barometric pressure 380 mm.*

|                     | MINUTE |    |    |    |    |     |     |     |
|---------------------|--------|----|----|----|----|-----|-----|-----|
|                     | 0      | 5  | 10 | 15 | 25 | 35  | 55  | 75  |
| Pulse.....          | 74     | 74 | 76 | 79 | 86 | 85  | 85  | 84  |
| Systolic.....       | 108    |    |    |    |    | 112 | 106 | 104 |
| Diastolic.....      | 76     |    |    |    |    | 70  | 70  | 66  |
| Pulse pressure..... | 32     |    |    |    |    | 42  | 36  | 38  |

|                         | MINUTE |          |     |     |           |     |     |
|-------------------------|--------|----------|-----|-----|-----------|-----|-----|
|                         | 0      | 8        | 14  | 18  | 23        | 71  | 82  |
| Respiration volume..... | 5.0    | 5.4      | 5.9 | 6.0 | 6.6       | 6.7 | 6.7 |
| Hemoglobin.....         | 100    | 42d, 108 |     |     | 76th, 110 |     |     |

The pulse rate and blood flow reached the maximum together about the 21st minute. The per-minute volume of breathing had increased by the 23rd minute, to the volume which was maintained throughout the holding period. The increase in hemoglobin did not favor either the circulation or respiration.

We believe that some members of this group failed to show an interplay between the hemoglobin, circulation and respiration because they were too near their critical low oxygen limit. The compensations were just able to meet the demand of the tissues for oxygen.

*c. Maximum pulse rate maintained without an increase in hemoglobin.* There were eight cases in this group. Several of the experiments will

be discussed in the study of repeated cases. It was to be expected that a failure in compensation by one mechanism might cause the others to hold a constant level when they had responded sufficiently to meet the demands of the body for oxygen. The following case is typical of the group.

*A. W. L. June 18, 1918. Barometric pressure 380 mm.*

|                     | MINUTE |     |     |     |     |     |     |     |
|---------------------|--------|-----|-----|-----|-----|-----|-----|-----|
|                     | 0      | 5   | 10  | 15  | 25  | 35  | 55  | 75  |
| Pulse.....          | 93     | 93  | 96  | 98  | 98  | 100 | 101 | 101 |
| Systolic.....       | 118    | 120 | 122 | 124 | 120 | 116 | 116 | 118 |
| Diastolic.....      | 76     |     |     |     | 78  | 64  | 56  | 60  |
| Pulse pressure..... | 42     |     |     |     | 42  | 52  | 60  | 58  |

|                                | MINUTE |      |      |      |      |      |      |      |      |
|--------------------------------|--------|------|------|------|------|------|------|------|------|
|                                | 0      | 10   | 15   | 25   | 45   | 55   | 65   | 75   | 85   |
| Alveolar O <sub>2</sub> .....  | 101.3  | 54.5 | 31.3 | 32.0 | 29.6 | 30.3 | 30.1 | 30.5 | 34.0 |
| Alveolar CO <sub>2</sub> ..... | 42.7   | 40.5 | 34.8 | 34.5 | 34.4 | 33.4 | 34.4 | 33.5 | 30.7 |

The rate of blood flow appeared to have reached a maximum about the 55th minute and then maintained the new level. The respiration also, after reaching its maximum value at the 25th minute, remained fairly constant until about the 80th minute, when it increased once more. There was no evidence of interplay of compensatory mechanisms throughout this experiment.

*d. Retardation in the pulse rate during the holding period with no increase in hemoglobin.* There were four cases in this group and the data for these is given below.

*F. C. P. December 8, 1918. Barometer 428 mm.*

|                                | MINUTE |    |      |      |      |    |      |      |      |
|--------------------------------|--------|----|------|------|------|----|------|------|------|
|                                | 0      | 5  | 10   | 15   | 25   | 35 | 42   | 50   | 75   |
| Pulse.....                     | 72     | 72 | 74   | 78   | 80   | 77 | 78   | 75   | 74   |
| Alveolar O <sub>2</sub> .....  | 97.1   |    | 58.1 | 44.2 | 44.6 |    | 38.6 | 49.3 | 38.6 |
| Alveolar CO <sub>2</sub> ..... | 39.7   |    | 36.7 | 32.8 | 30.3 |    | 31.9 | 25.5 | 31.4 |

This experiment was conducted at a barometric pressure of 428 mm. (15,000 feet). The breathing as shown by the alveolar carbon dioxide was variable. The carbon dioxide tension was lowest at the 25th and



50th minutes. The high alveolar oxygen tension at the 50th minute was sufficient to account for the falling off in pulse rate.

*C. P. C. December 13, 1918. Barometric pressure 428 mm.*

|                                | MINUTE |      |      |      |    |    |    |      |
|--------------------------------|--------|------|------|------|----|----|----|------|
|                                | 0      | 5    | 10   | 15   | 25 | 35 | 45 | 58   |
| Pulse .....                    | 84     | 88   | 94   | 98   | 95 | 92 | 88 |      |
| Alveolar O <sub>2</sub> .....  | 96.0   | 84.5 | 56.0 | 41.6 |    |    |    | 48.4 |
| Alveolar CO <sub>2</sub> ..... | 32.2   | 22.6 | 23.3 | 20.4 |    |    |    | 18.7 |

The respiration increased throughout the entire period which probably accounts for the slowing of the pulse rate.

F. D., January 13, 1919, was subjected to a barometric pressure of 395 mm. (17,000 feet) in an experiment which lasted 100 minutes. The hemoglobin did not increase. The pulse rate accelerated from 72 to 99 by the 15th minute. It held this rate for three minutes, was 90 at the 25th minute and 78 at the 70th minute, where it remained until the close of the experiment. The respiration was not measured, but the observer and the subject noticed that the subject's breathing increased and became labored at the 25th minute, and remained so until the end.

*H. J. M. June 20, 1918. Barometric pressure 380 mm.*

|                      | MINUTE |    |    |    |     |     |     |     |
|----------------------|--------|----|----|----|-----|-----|-----|-----|
|                      | 0      | 5  | 10 | 15 | 25  | 35  | 55  | 63  |
| Pulse .....          | 80     | 83 | 89 | 93 | 100 | 97  | 92  | 100 |
| Systolic .....       | 108    |    |    |    |     | 112 | 114 |     |
| Diastolic .....      | 64     |    |    |    |     | 48  | 48  |     |
| Pulse pressure ..... | 44     |    |    |    |     | 64  | 66  |     |

|                          | MINUTE    |     |     |     |     |     |     |     |
|--------------------------|-----------|-----|-----|-----|-----|-----|-----|-----|
|                          | 0         | 18  | 21  | 24  | 33  | 42  | 57  | 66  |
| Respiration volume ..... | 5.4       | 5.6 | 6.7 | 6.0 | 6.3 | 6.3 | 5.8 | 6.6 |
| Hemoglobin .....         | No change |     |     |     |     |     |     |     |

A definite fall in the pulse rate began at the 46th minute and lasted until the 56th minute, after which it gradually accelerated again. There is nothing that accounts for this fall in pulse rate. The arterial pressures were not taken often enough to make an interpretation of the blood flow changes.

We believe that our data accounts, in three out of four of the cases, for the decrease in pulse rate that occurred during the holding period. The interplay in these cases was between the respiratory and circulatory compensations to low oxygen.

#### REPEATED EXPERIMENTS ON ONE INDIVIDUAL

Five men served as subjects from two to five times each. The data in four cases are complete enough to make comparisons worth while.

*W. H. G. May 24, 1918. Barometric pressure 428 mm.*

|                     | MINUTE |     |     |     |     |     |     |
|---------------------|--------|-----|-----|-----|-----|-----|-----|
|                     | 0      | 5   | 10  | 15  | 25  | 35  | 55  |
| Pulse .....         | 76     | 82  | 85  | 94  | 93  | 91  | 90  |
| Systolic.....       | 100    | 102 | 102 | 102 | 104 | 104 | 100 |
| Diastolic.....      | 66     |     |     | 60  | 62  | 66  | 68  |
| Pulse pressure..... | 34     |     |     | 42  | 42  | 38  | 32  |

|                                | MINUTE |      |      |      |      |      |      |
|--------------------------------|--------|------|------|------|------|------|------|
|                                | 0      | 10   | 15   | 25   | 35   | 45   | 65   |
| Alveolar O <sub>2</sub> .....  | 106.8  | 72.5 | 50.8 | 45.2 | 48.8 | 43.7 | 46.7 |
| Alveolar CO <sub>2</sub> ..... | 37.3   | 33.0 | 30.4 | 31.7 | 28.9 | 31.1 | 28.3 |
| Hemoglobin.....                | 96     |      | 103  | 106  | 106  | 105  | 105  |

*W. H. G. July 10, 1918. Barometric pressure 380 mm.*

|                     | MINUTE |     |     |     |     |     |     |     |     |
|---------------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|
|                     | 0      | 5   | 10  | 15  | 25  | 35  | 55  | 75  | 95  |
| Pulse.....          | 78     | 82  | 90  | 97  | 100 | 98  | 96  | 93  | 94  |
| Systolic.....       | 110    | 110 | 108 | 114 | 120 | 116 | 116 | 114 | 110 |
| Diastolic.....      | 70     |     |     |     | 68  | 68  | 70  | 64  | 64  |
| Pulse pressure..... | 40     |     |     |     | 52  | 48  | 46  | 50  | 44  |

|                         | MINUTE |           |     |     |           |     |     |           |     |     |
|-------------------------|--------|-----------|-----|-----|-----------|-----|-----|-----------|-----|-----|
|                         | 0      | 6         | 12  | 21  | 27        | 39  | 42  | 60        | 75  | 102 |
| Respiration volume..... | 5.7    | 6.3       | 6.6 | 8.1 | 7.8       | 7.6 | 6.6 | 6.5       | 6.8 | 6.7 |
| Hemoglobin.....         | 98     | 46th, 101 |     |     | 65th, 101 |     |     | 95th, 104 |     |     |

In both experiments the blood flow, as shown by the pulse rate and the pulse pressure, reached its maximum immediately after the low barometric pressure was attained. An increase in hemoglobin was

observed in each experiment when the pulse rate began to retard. The respiration, in the experiment in which the barometric pressure was 428 mm., increased during the ascent, then maintained a level. In the experiment at 380 mm. the respiratory per-minute volume was increased from 5.7 to 8.1 liters during the ascent. It then decreased slowly to 6.6 liters at the 42nd minute, after which it remained constant. In the first experiment the increase in hemoglobin spared the circulation, in the second both circulation and respiration shared the gain.

B. R. L. was taken twice to 380 mm. The results are tabulated below.

*B. R. L. August 5, 1918*

|                              | MINUTE |           |      |           |      |            |      |           |      |
|------------------------------|--------|-----------|------|-----------|------|------------|------|-----------|------|
|                              | 0      | 5         | 10   | 15        | 25   | 35         | 55   | 75        | 95   |
| Pulse.....                   | 84     | 85        | 85   | 88        | 90   | 86         | 84   | 82        | 83   |
| Systolic.....                | 104    | 102       | 100  | 102       |      |            | 92   | 98        | 102  |
| Diastolic.....               | 62     |           |      | 62        |      |            | 60   | 66        | 64   |
| Pulse pressure.....          | 42     |           |      | 40        |      |            | 32   | 32        | 38   |
| Alveolar O <sub>2</sub> .... | 107.3  |           | 75.0 | 44.1      | 42.2 | 44.0       | 48.0 | 50.0      | 43.2 |
| Alveolar CO <sub>2</sub> ..  | 37.3   |           | 30.9 | 27.4      | 29.6 | 25.0       | 22.9 | 19.8      | 23.4 |
| Hemoglobin....               | 107    | 45th, 111 |      | 60th, 112 |      | 100th, 112 |      | 142d, 116 |      |

*B. R. L. May 12, 1919*

|                     | MINUTE |    |    |    |    |     |    |     |    |
|---------------------|--------|----|----|----|----|-----|----|-----|----|
|                     | 0      | 5  | 10 | 15 | 22 | 25  | 35 | 55  | 66 |
| Pulse.....          | 85     | 88 | 94 | 94 | 86 | 86  | 88 | 88  | 82 |
| Systolic.....       | 110    |    |    |    |    | 110 |    | 112 |    |
| Diastolic.....      | 70     |    |    |    |    | 70  |    | 68  |    |
| Pulse pressure..... | 40     |    |    |    |    | 40  |    | 44  |    |

|                                | MINUTE |           |      |          |           |           |
|--------------------------------|--------|-----------|------|----------|-----------|-----------|
|                                | 0      | 5         | 23   | 33       | 46        | 59        |
| Alveolar O <sub>2</sub> .....  | 104.4  | 90.0      | 49.3 | 48.3     | 51.6      | 52.0      |
| Alveolar CO <sub>2</sub> ..... | 39.1   | 33.6      | 31.4 | 19.5     | 18.3      | 18.3      |
| Hemoglobin .....               | 100    | 25th, 106 |      | 43d, 106 | 88th, 107 | 90th, 107 |

The two experiments, while separated by nine months, were quite similar and unusual in several respects. In both the pulse reached its maximum rate quickly and returned to normal or subnormal before the close of the experiment. The respiratory increase was more marked

than in the usual case, in that the carbon dioxide instead of falling to the average figure of 31 mm., reached 19.8 mm. in the first and 18.3 mm. in the second experiment. A good increase in ventilation occurred during the ascent and it continued to increase for some time after the barometric pressure was maintained at 380 mm. The hemoglobin increased 8.4 per cent in one and 8 per cent in the other. It should be noted that in each experiment the compensation was made at first by the circulation and respiration, but later it was borne wholly by the respiration and hemoglobin, in that the pulse rate slowed to normal or subnormal.

A. F. H. served as a subject four times and did not tolerate the low oxygen tensions equally well each time. The data are summarized in the following protocols.

*A. F. H. July 8, 1918. Barometric pressure 380 mm.*

|                |  | MINUTE |    |    |    |    |    |    |    |
|----------------|--|--------|----|----|----|----|----|----|----|
|                |  | 0      | 5  | 10 | 15 | 25 | 35 | 55 | 75 |
| Pulse.....     |  | 72     | 72 | 75 | 87 | 88 | 87 | 84 | 84 |
| Systolic.....  |  | 104    |    |    |    |    |    | 94 |    |
| Diastolic..... |  | 70     |    |    |    |    |    | 66 |    |

|                    |     | MINUTE    |     |           |     |     |           |     |     |     |    |
|--------------------|-----|-----------|-----|-----------|-----|-----|-----------|-----|-----|-----|----|
|                    |     | 0         | 15  | 18        | 21  | 24  | 42        | 45  | 54  | 75  | 87 |
| Respiration volume | 6.7 | 6.2       | 8.4 | 9.1       | 9.4 | 9.4 | 8.3       | 7.9 | 7.1 | 6.0 |    |
| Hemoglobin.....    | 99  | 40th, 100 |     | 60th, 104 |     |     | 86th, 104 |     |     |     |    |

*A. F. H. July 15, 1918. Barometric pressure 380 mm.*

|                     | MINUTE |     |    |    |     |     |     |     |
|---------------------|--------|-----|----|----|-----|-----|-----|-----|
|                     | 0      | 5   | 10 | 15 | 25  | 35  | 55  | 75  |
| Pulse .....         | 69     | 69  | 72 | 72 | 78  | 81  | 79  | 72  |
| Systolic.....       | 106    | 100 | 98 | 98 | 100 | 104 | 102 | 108 |
| Diastolic.....      | 70     |     |    |    | 70  |     |     | 72  |
| Pulse pressure..... | 36     |     |    |    | 30  |     |     | 36  |

|                      | MINUTE |          |     |          |     |          |     |     |      |      |      |
|----------------------|--------|----------|-----|----------|-----|----------|-----|-----|------|------|------|
|                      | 0      | 15       | 18  | 21       | 24  | 42       | 45  | 54  | 60   | 66   | 75   |
| Respiration volume.. | 7.6    | 7.7      | 8.2 | 9.7      | 9.3 | 10.2     | 7.8 | 8.7 | 11.4 | 15.5 | 19.1 |
| Hemoglobin.....      | 94     | 40th, 95 |     | 60th, 95 |     | 78th, 98 |     |     |      |      |      |



*A. F. H. July 30, 1918. Barometric pressure 380 mm.*

|                     | MINUTE |    |     |     |     |     |    |    |
|---------------------|--------|----|-----|-----|-----|-----|----|----|
|                     | 0      | 5  | 10  | 15  | 25  | 35  | 55 | 65 |
| Pulse.....          | 78     | 81 | 81  | 86  | 86  | 87  | 90 | 93 |
| Systolic.....       | 112    |    | 112 | 108 | 108 | 108 | 96 |    |
| Diastolic.....      | 68     |    | 62  | 70  | 72  | 62  | 58 |    |
| Pulse pressure..... | 44     |    | 50  | 38  | 36  | 46  | 38 |    |

Respiration not taken.

Hemoglobin: No change.

A. F. H. apparently tolerated a barometric pressure of 380 mm. better on July 8 than during the later exposures. During the first experiment the pulse reached its maximum rate, 90, at the 18th minute. It slowed at about the time the hemoglobin increased. The per-minute volume of breathing showed an early and marked increase which reached its maximum at the 24th minute and held until the 42nd, after which it returned to the pre-experimental volume. The respiration especially and the circulation slightly seemed to have been favored by the increase in hemoglobin toward the latter part of this experiment.

In the second experiment the demand for oxygen was met by an entirely different compensatory reaction. The pulse reached its maximum rate slowly, at the 31st minute, and then remained more or less constant until the 59th minute, when it decreased almost to the pre-experimental rate as the respiration increased. The respiration reached a first high point at the 21st minute, which it held until the 42nd minute. It then decreased for six minutes. At this time a great and progressive increase in breathing began which finally changed the per-minute volume from 7.6 to 19.8 liters. The association of the pulse rate and the respiration was conspicuous in this experiment. The part played by the hemoglobin is obscured by the respiratory response.

In the third experiment, July 30, the respiration was not recorded. The hemoglobin did not increase, and the pulse rate gradually accelerated from 78 to 93 throughout the period of experimentation. This subject had made frequent ascents in the low pressure chamber. He felt more uncomfortable this time than in any previous experiment. He noticed a blurring of vision which had never occurred before.

On May 21, 1918, this subject was taken to a barometric pressure of 425 mm. in 15 minutes, held there for 4 minutes, taken down to 700 mm., held there for about 5 minutes, then taken again to 425 mm. and kept there for 30 minutes. In this experiment the respiratory volume

increased and decreased with the barometer. The pulse accelerated in the first ascent from 74 to 96, then dropped to 70 and accelerated to 82 in the second. The hemoglobin increased from 94 to 98, 4.3 per cent.

These four experiments with A. F. H. show clearly that an individual does not necessarily use the three compensatory mechanisms in equal degree each time he encounters low oxygen tension. It is evident that the burden of compensation may be met adequately in several ways. It appears also that the compensatory changes at a particular pressure may be adequate on some occasions and inadequate at other times. It is probable that A. F. H., if held a little longer at 380 mm., would have developed a typical case of altitude sickness.

W. B. M. served as a subject five times. In the first experiment he was taken to a barometric pressure of 425 mm. and in the others to 380 mm. At 425 mm. the pulse accelerated from 63, reaching its maximum rate, 80, seven minutes after 425 mm. was attained. It held that rate until the 68th minute when it decreased to 76 and remained constant. The hemoglobin gave no evidence of concentration up to the 55th minute, but from the 55th to the 75th minutes it increased from 104 to 110 per cent. The alveolar air showed that the maximum ventilation of the lungs was reached at the 15th minute, after which it took a lower level which was maintained until the end. The compensations were good in this experiment and the interplay of the compensatory factors was evident.

At 380 mm., in the experiments of June 12, 28 and July 31, the pulse after reaching a maximum rate did not fall definitely. The pulse rate in each of the cases showed fluctuations lasting from 5 to 10 minutes. In these the rate retarded at first and then accelerated to the previous high level. The hemoglobin increased during the experiments of June 12 and 28 but did not spare either the respiration or the circulation. In both cases the respiration responded slowly, requiring 42 and 70 minutes to reach the maximum. In the experiment of July 31, the respiration was not studied. The hemoglobin failed to show concentration. The pulse rate and the blood flow reached their maxima shortly after 380 mm. was reached and then maintained that level for 56 minutes, or until the end of the experiment. This subject was again under observation at 380 mm. about five months later, December 27. His pulse at this time accelerated from a rate of 69 at the beginning to 110 at the 28th minute, then quickly retarded to 100 and from this point fell slightly toward the end of the experiment. The pulse showed the same fluctuations in rate as were observed in the earlier experiments

with this subject. The hemoglobin increased 8.8 per cent. We are inclined to believe that a pressure of 380 mm. was too low for this subject. At 428 mm. his compensations were adequate and gave opportunity for an increase in hemoglobin to spare the other factors. At 380 mm. slight movements caused a temporary upset in the balance of the compensatory factors.

The data presented show clearly that on exposure to a decreasing barometric pressure the circulatory and respiratory mechanisms are both stimulated to increased activity. Thus far we have been unable to determine which of these two mechanisms is most sensitive to the change. In many men the heart responded by an acceleration in the rate of beat, and the per-minute volume of breathing increased at about the same time and at barometric pressures that corresponded to relatively low altitudes (4000 feet). Usually the first evidence of response occurred at a higher altitude. Sometimes the pulse rate accelerated before the breathing increased and vice versa. The degree of response made by these two mechanisms is shown to vary with individuals. Thus during the ascent to 380 mm. the pulse rate accelerated from 5 to 30 beats. The volume of breathing also showed corresponding differences.

When a desired pressure was reached and maintained, these mechanisms continued to show differences. The ventilation of the lungs in some men became maximal during the ascent, in others a few minutes after arrival at the constant pressure, while in a few it increased slowly throughout the entire period. The maximum ventilation was likewise maintained for a few minutes, a considerable portion of the time, or for the entire period of the constant pressure. Often after a period of maximal breathing the per-minute ventilation of the lungs was somewhat reduced. In one experiment with A. F. H. it returned to the pre-experimental volume. The pulse rate and blood flow showed a similar variety of changes.

The hemoglobin always increased slowly. In some men no increase could be detected, in others it increased as much as 10 per cent. In a few men the increase began as early as 25 minutes, usually between the 40th and 60th minutes, sometimes as late as the 75th minute. Usually the pulse rate decreased while the hemoglobin increased. Sometimes the pulse rate and the breathing decreased, and in a few instances only the breathing decreased as the hemoglobin concentrated.

The data presented show that the early compensations are made exclusively by the circulatory and respiratory mechanisms. Later

the increase in red corpuscles and hemoglobin shared the burden with the circulatory and respiratory mechanisms. When the early compensation was adequate, as it appears to have been in most cases, the increase in hemoglobin caused a falling off in the activity of either the circulation or the respiration, or both. When the compensation was not adequate, the increase in hemoglobin failed to relieve the other mechanisms to any extent.

That an individual may make equal use of the adaptive mechanisms during several exposures to low barometric pressures is indicated by the repeated experiments on W. H. G., B. R. L. and W. B. M. As illustrated in the four experiments with A. F. H., the responses may differ during two ascents. These experiments show that it is impossible to predict with exactness just how a given individual will react to low oxygen during exposures from 30 to 120 minutes. The three factors of compensation are capable of a variety of combinations. The normal response to low oxygen makes use of the circulatory, respiratory and blood changes.

#### EXPERIMENTS AT ATMOSPHERIC PRESSURE WITH 10 PER CENT OXYGEN

In this group of experiments the subjects breathed atmospheric air diluted with nitrogen. Starting with undiluted air the nitrogen was added gradually until the proportion gave a mixture that gave 10 per cent oxygen at the end of 20 minutes. This percentage was then maintained. The hemoglobin changes were studied in seven experiments. Three of these gave no increase in hemoglobin and also failed to show a falling off in pulse rate during the period of maintained constant percentage of oxygen. The cases in which an increase in hemoglobin occurred are discussed below.

*G. B. H. May 28, 1918. To 10 per cent oxygen in 20 minutes*

|                     | MINUTE |     |           |     |          |     |     |     |
|---------------------|--------|-----|-----------|-----|----------|-----|-----|-----|
|                     | 0      | 5   | 10        | 15  | 25       | 35  | 55  | 75  |
| Pulse.....          | 88     | 90  | 92        | 95  | 103      | 102 | 98  | 102 |
| Systolic.....       | 110    | 112 | 112       | 110 | 112      | 116 | 110 | 104 |
| Diastolic.....      | 70     | 70  | 70        | 66  | 66       | 64  | 62  | 54  |
| Pulse pressure..... | 40     | 42  | 42        | 44  | 46       | 52  | 48  | 50  |
| Hemoglobin.....     | 100    |     | 70th, 100 |     | 82d, 104 |     |     |     |



The respiration was recorded by means of a Fitz pneumograph. It showed a definite increase at the 18th minute with a progressive increase to the 38th minute, and then a plateau until the 48th minute. After this a definite falling off in respiration occurred. The pulse rate accelerated until the 20th minute and then maintained a more or less constant level. The blood flow as judged from the pulse pressure reached its maximum about the 35th minute and then held. The increase in hemoglobin came late. The diminution in respiration began before the concentration in hemoglobin was detected. Hence the evidence of an interplay of compensatory factors is uncertain.

W. A. B. gave an excellent response. His data have been plotted in figure 3. The pulse rate reached its maximum about the 20th minute, which was the period of maximum blood flow as indicated by the pulse pressure. The respiration, which was recorded by means of a pneumograph, reached its maximum at about the same time, the 25th minute, and held there until the 38th minute. After this the per-minute volume of breathing decreased somewhat. The hemoglobin had begun to increase at the 22nd minute. In this case the concentration in hemoglobin appears to be definitely related to a diminution in respiration and a decrease in pulse rate.

*W. O. K. June 3, 1918. To 10 per cent oxygen in 20 minutes*

|                   | MINUTE |           |     |           |     |           |     |     |
|-------------------|--------|-----------|-----|-----------|-----|-----------|-----|-----|
|                   | 0      | 5         | 10  | 15        | 25  | 35        | 40  | 48  |
| Pulse.....        | 72     | 75        | 78  | 78        | 78  | 76        | 78  | 86  |
| Systolic.....     | 110    | 108       | 108 | 112       | 108 | 112       | 112 | 114 |
| Diastolic.....    | 78     | 80        | 80  | 78        | 80  | 78        | 70  | 68  |
| Pulse pressure... | 32     | 28        | 28  | 34        | 28  | 34        | 42  | 56  |
| Hemoglobin.....   | 100    | 15th, 102 |     | 39th, 106 |     | 50th, 108 |     |     |

The respiration as shown by a pneumograph tracing was somewhat excessive at the start. It quieted later and maintained a constant level until the 48th minute when it suddenly became labored. The depth at this time increased to three times its former value while the rate remained unchanged. There was no evidence of an interplay of the compensatory factors in this experiment. Ten per cent oxygen was too low for this subject, since he fainted at the 51st minute. In a low barometric pressure experiment, at 428 mm., his pulse maintained its maximum rate throughout even though there was a progressive increase in lung ventilation. There was no evidence of an interplay of factors in the experiment.

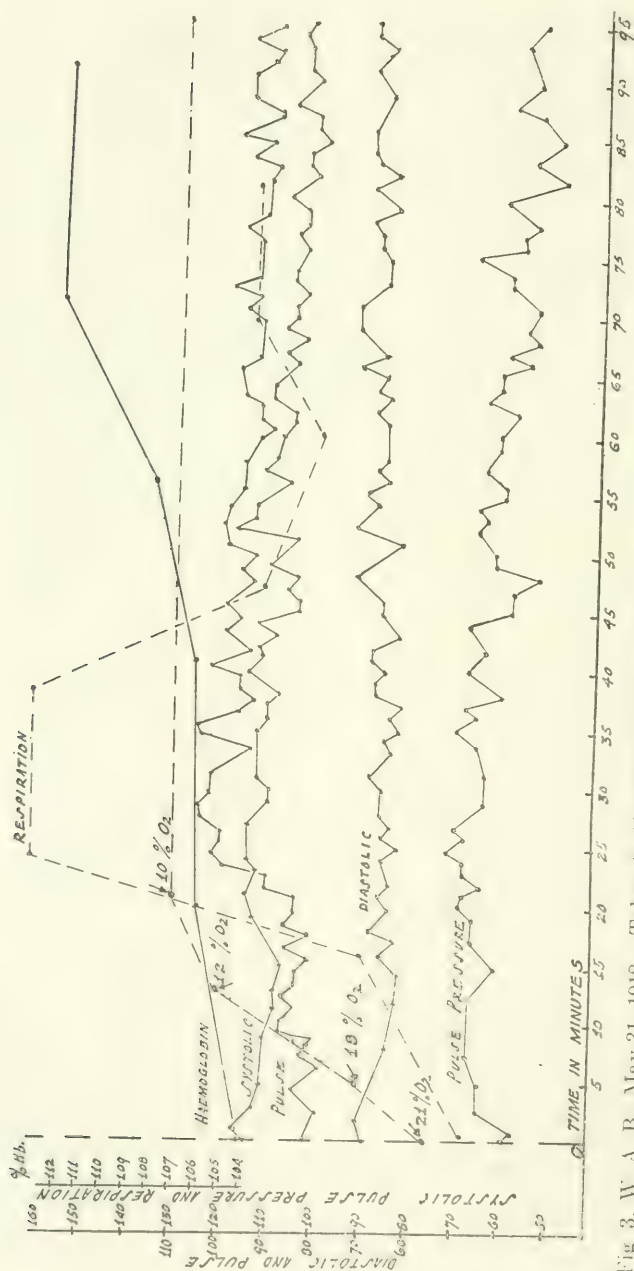


Fig. 3. W. A. B., May 31, 1918. Taken to 10 per cent oxygen (19,400 feet) in 21 minutes by the nitrogen dilution method. The interplay of blood flow, respiration and hemoglobin is shown. Compare with figures 1 and 2.

E. A. R., in an experiment similar to those just described, gave a 5 per cent increase in hemoglobin which began about the 20th minute. The pulse rate accelerated from 68 to 78 in 32 minutes and then held. The respiration was not recorded. Interplay between the hemoglobin and the circulation was lacking unless it be considered that the early concentration of hemoglobin made it unnecessary for the pulse rate to increase as much as in the other cases. In a low barometric pressure experiment, 380 mm., this subject showed a greater increase in the pulse rate. It accelerated from 72 to 93 and later retarded to 86. His per-minute ventilation increased from 7.8 to 10 liters and then decreased as the hemoglobin concentrated.

We are of the opinion that these experiments were made at too low an oxygen percentage to obtain the optimum response. Ten per cent oxygen, which was maintained during the holding period, is equivalent to an altitude of 19,400 feet. Few men could remain long at such an altitude and escape altitude sickness. Under these circumstances the response made by W. A. B. gives striking confirmation to the view that the effects of low barometric pressure and low percentage of oxygen are due to the same cause, namely, a low partial pressure of oxygen. It also proves that an interplay of the compensatory factors may occur in men subjected to low percentages of oxygen.

We shall not enter here into a discussion of the mechanisms by which the changes observed in these experiments are produced. We desire to point out how the reactions which we have been studying differ from those that occur in men residing at high altitudes. The acclimatization to oxygen want seen in men living at high altitudes involves the same mechanisms that we find in the compensation during a rapid lowering of oxygen tension and comparatively short exposures to low oxygen. Ordinarily on ascending a mountain passively, by railway or automobile, the respiratory response is the first to appear, beginning during the ascent or almost immediately after the summit is reached. It requires, however, several weeks for the respiration to increase to the volume that is normal for the new altitude (5). The blood does not show immediately the increase in hemoglobin and red corpuscles. Just when these changes begin has not been determined, but usually within 24 hours a marked increase in both can be observed. They require five or more weeks to reach their greatest concentration. The pulse also does not ordinarily accelerate immediately but the rate increases slowly during a period of several days. The changes in the breathing and in the blood are permanent in character and do not diminish during

a protracted residence at the high altitude. The changes in the pulse rate and in the rate of blood flow are of a less permanent character. With acclimatization the pulse rate returns somewhat toward the normal rate at sea level. It has been shown also that the longer the period of sojourn at a high altitude the more enduring are the after-effects when the subject again descends to a low altitude. The permanence of these changes has been attributed to diminished alkalinity of the blood, to permanent alteration in the exciting threshold of blood reaction for the kidneys, or to other changes of a more or less permanent character.

The compensations which we have presented in this paper are quick to develop and temporary in character. They disappear at once, or at least quickly, when ordinary atmospheric pressure and oxygen tensions are restored. That they were never quite sufficient at a barometric pressure of 380 mm. was indicated by the fact that while cyanosis often improved when a low oxygen level was maintained, it never disappeared entirely. Furthermore some men who appeared to be compensating well lost gradually in mental efficiency or became abnormally sleepy. It should also be noted that as experience on mountains has demonstrated, if the experiments had been continued several hours longer, the majority of our subjects would have developed typical cases of altitude sickness. Headache and fatigue were often observed as after-effects.

The differences in the responses observed under these two different conditions of exposure to low oxygen depend no doubt upon the suddenness with which the low barometric pressure and low oxygen percentage have been decreased and upon the extent to which they were lowered. In very slow and moderate changes it is possible that no response may be evoked. Possibly the respiratory center, by virtue of greater sensitiveness, may react so much to the stimulus that the increase in respiration for a time cares adequately for the oxygen requirement of the body. In the more rapid decrease in oxygen tension the respiratory and cardiac centers and very likely the vasomotor centers are stimulated at higher oxygen tensions and at about the same time. Consequently under the conditions of our experiments these two mechanisms served almost equally to care for the oxygen need of the body.



## SUMMARY

1. During a period of gradual reduction in oxygen partial pressure, at a rate approximately 5 mm. per minute, the respiratory and cardiac centers are ordinarily stimulated by about the same fall in the oxygen pressure. In some subjects the first response began at an oxygen partial pressure of 147 mm., in the majority between 128 and 113 mm. In some men the circulation responded before the respiration, and in others the order was reversed. The compensations during the period of reduction, which lasted 15 to 20 minutes, were made entirely by the circulation and respiration.

2. The compensations during an exposure to a constant low oxygen tension were classified as follows: *a*, Those in which the pulse, after maintaining a high rate for a while, retarded slowly and the percentage of hemoglobin increased; *b*, those in which the pulse after a primary rise maintained a constant rate while the hemoglobin increased; *c*, those in which the pulse rate after the primary rise remained constant and in which the hemoglobin did not increase; *d*, those in which the pulse rate after a primary rise retarded and the hemoglobin did not increase. The compensations were distributed among these groups as follows: *a*, 55 per cent; *b*, 19 per cent; *c*, 17 per cent; *d*, 9 per cent.

3. During an exposure of 30 to 145 minutes to low oxygen tension the percentage of hemoglobin usually increased in 20 minutes or more. When this occurred, and when the compensation which had been made by the respiratory and circulatory systems was adequate, the circulation or the respiration, or both, decreased as the hemoglobin increased. In several cases the sparing action of the hemoglobin restored the pulse rate to normal, that is, the pre-experimental rate, and in one case the respiration returned to normal.

4. The interdependence of the three compensatory reactions was shown also in a few cases in which an increase in breathing, following a period of equilibrium in circulation and respiration, resulted in a retardation in pulse rate and blood flow.

5. Several individuals compensated in the same manner and in about equal degree in two or more experiments. One man compensated differently in each of four experiments.

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# THE REACTIONS OF THE CARDIAC AND RESPIRATORY CENTERS TO CHANGES IN OXYGEN TENSION

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The fact that the medullary centers of the brain in man are very sensitive to changes in available oxygen became apparent early in the rebreathing and low pressure experiments conducted at the Medical Research Laboratory of the Air Service, Mineola, New York. We (1) have shown that the respiratory and cardiac centers are stimulated to increased activity in some men when the atmospheric oxygen tension is decreased from 159 to 137 mm. Hg. in about four minutes. The respiratory response at first is an increase in the depth but not in the rate of breathing. The cardiac response is shown by an acceleration in the pulse rate. Because these responses developed before the organism appeared to come under stress from lack of oxygen we became interested in attempting to determine how quickly and to what extent the breathing and the rate of heart beat will respond to sudden changes in oxygen tension.

In the past, asphyxia and low oxygen effects have been studied in animals in considerable detail. Paul Bert (2) working with man and animals showed in 1878 that when oxygen was administered at a low barometric pressure in a pneumatic cabinet, the pulse rate retarded soon after the inhalation of oxygen was begun. Traube (3) in 1863 investigated the action of asphyxia upon curarized rabbits. The earlier experiments did not distinguish clearly between the effects due to lack of oxygen and those due to the accumulation of carbon dioxide. The complete separation of the two conditions by the use of such gases as hydrogen, nitrogen or carbon monoxide has proved that the initial effect of oxygen lack on the medullary centers is clearly stimulating (4). Loevenhart has written,

The striking symptoms of asphyxia, however produced, are the following: increase in the rate and depth of the respiration, rise of blood pressure, slowing of the pulse, cessation of respiration, general convulsions followed by paralysis, marked and progressive fall in blood pressure, death.

Gasser and Loevenhart (6) found that decreased oxidation stimulates the medullary centers in the following order: respiratory, vasomotor and cardio-inhibitory, and that on further decrease in oxygen the centers become depressed and finally paralyzed in the order named. Loevenhart has omitted from his list of symptoms of asphyxia the acceleration of the heart which has often been recorded. Lewis and Mathison (7) observe that the heart accelerates within two or three minutes of the onset of asphyxia.

The majority of researches have dealt in detail only with the late and more acute conditions of the reactions to asphyxia and anoxemia. Gasser and Loevenhart (6), however, have determined the latent periods for the action of decreased oxygen on rabbits, dogs and cats by the administration of carbon monoxide and sodium cyanide. The latent periods calculated from the beginning of the administration ranged for the respiratory center between 4 and 14.5 seconds, averaging 6 or 7 seconds, and for the cardio-inhibitory center between 15 and 50 seconds.

In our experiments we have studied men and have confined our attention to the early effects on the heart rate and on the respiration of a reduced oxygen supply. We have also studied the opposite condition in which either oxygen or normal air was given after the individual showed clearly the effects of oxygen want, and we have recorded the heart rate and respiration under these conditions.

#### METHOD

The effects of anoxemia and the restoration of normal oxygen tension on the pulse rate and respiration were studied by having men breathe pure nitrogen. Nitrogen, saturated with water vapor at room temperature, was supplied through Larsen's spirometer from a rubber bag containing about 80 liters. In a few cases the subjects inspired directly from the bag and exhaled into the room. The Larsen apparatus was used, however, because it gave an opportunity to measure the volume of each breath. Before the experiment the subject sat quietly with a mouth-piece in place but breathing through his nose. Simultaneous records of the pulse and respiration rate and amplitude were taken by means of a Mackenzie polygraph to which a Fitz pneumograph was connected. After sufficient normal record had been secured, with the apparatus still recording, the nose clip was put on and the spirometer opened at the same time so that the subject began to breathe nitrogen without interrupting the record. When the pulse and respi-



ration became markedly increased and the subject began to appear ashy pale, the pupils dilated and unconsciousness impending, the nose clip was removed and the spirometer closed so that the subject breathed atmospheric air without interrupting the pulse and respiratory record, which was continued in most cases until the normal was resumed. No cases of fainting occurred, but it was evident, when the method was being tried out on ourselves, that unconsciousness could come on without fainting, and a few of our subjects were taken to this point.

From the continuous record the pulse was counted for five-second periods throughout. The length of each respiration was measured in seconds and recorded as the rate per minute. The amplitude of each respiration was measured in millimeters. The volume of each breath of nitrogen was read in deciliters from the dial of the respirometer. Unfortunately the return of the per-minute volume, when air was given, could not be followed with the apparatus, but the amplitude of each breath during this period was recorded. In the pulse studies in the low pressure chamber a continuous pulse tracing was taken with the polygraph covering the period before, during and after the taking of oxygen from the tube. In cases in which the effect of oxygen on respiration at low oxygen tension was studied, the Larsen spirometer was used in the low pressure chamber, and at the desired moment oxygen was allowed to fill the spirometer.

The excitability of the medullary centers to changes in the partial pressure of oxygen was determined in two ways; first, by a reduction in available oxygen, and second, by a sudden increase in the oxygen obtained by returning the subject quickly to atmospheric air or by the administration of pure oxygen. By the first method the centers were stimulated and by the second their activity was diminished.

For the determination of the latent period the second method was found to give more uniform results than were obtained by decreasing the oxygen supply. The nitrogen experiments gave the best illustration of this difference. Many of our subjects showed anxiety which was manifested in a high pulse rate and slightly increased breathing. These conditions naturally masked the onset of the stimulating action of the lowered oxygen tension. A further cause for the variation in the length of the latent period was found in the depth of breathing. In passing from the breathing of ordinary atmospheric air to pure nitrogen the depth of breathing was usually so shallow that the amount of nitrogen that passed the dead space of the lungs was comparatively small. It frequently required two or three breaths to alter profoundly the alveolar

oxygen. When the lungs were well filled with nitrogen, the breathing became deep and rapid. Under these circumstances when oxygen was given or the subject was returned to atmospheric air, the first breath because of its depth carried a large amount of oxygen into the alveoli, and since the breathing was also more rapid, a second large influx of oxygen quickly followed the first.

#### PULSE RATE

The pulse rate data for the nitrogen experiments have been tabulated in table 1. In order that the pulse counts for the entire period might be presented, we have recorded the rate in ten-second intervals. From the polygraph tracings we have taken as our unit five seconds rather than ten seconds. The latent period has been calculated from the beginning of the administration of nitrogen in the study of the effects of decrease in oxygen, and from the time the subject was returned to atmospheric air or given oxygen for the determination of the time of beneficial effects of oxygen. In each case the interpretation consisted of determining in which five seconds the pulse rate had definitely changed in the proper direction, and then of recording the interval that had elapsed up to this five-second period as the length of the latent period. The following are typical experiments with nitrogen in which the pulse rate is recorded in intervals of five seconds.

|           | BEFORE<br>NITROGEN | NITROGEN ON           | NITROGEN OFF        | TIME ON        |
|-----------|--------------------|-----------------------|---------------------|----------------|
|           |                    |                       |                     | <i>seconds</i> |
| J. D..... | 6,6,6,7,6,7        | 7,8,9,9,9,9,9         | 9,9,7,8,6,6,7,6     | 42             |
| W. B..... | 6,7,6,6,6,6        | 6,7,8,7,8,9,10,11,10  | 11,10,9,8,7,8,7,6,6 | 45             |
| C. L..... | 8,8,8,8,9,8        | 8,8,8,9,9,11,11,11,11 | 12,11,10,9,8,8,8    | 46             |

The latent period for the stimulating effect of a decrease in oxygen during the breathing of nitrogen ranged between 5 and 55 seconds. Approximately 44 per cent of all cases gave a latent period of not more than 10 seconds, and 22 per cent gave one of 15 seconds. Thus a total of approximately 66 per cent of all experiments showed a latent period of 15 seconds or less.

The latent period determined for the opposite action, namely, an increase in oxygen percentage in which the pulse rate was retarded, ranged between 5 and 30 seconds. In about 45 per cent of all cases the latent period was 10 seconds or less, and in 41 per cent between 10

TABLE 1

*Effects of breathing nitrogen on the pulse rate. Rates calculated from 10 second periods on the sphygmogram. First heavy line indicates time on, second indicates time off*

| NUM-<br>BER | 10  | 20  | 30  | 10  | 20  | 30  | 40  | 50  | 60  | 70  | 80  | 90  | 100 | 110 | 120 | 130 | TIME<br>ON |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------------|
| 1           | 72  | 72  | 78  | 72  | 72  | 78  | 78  | 84  | 90  | 102 | 114 | 90  | 90  | 72  | 72  |     | 71         |
| 2           | 78  | 72  | 84  | 84  | 84  | 90  | 96  | 96  | 102 | 96  | 96  | 78  |     |     |     |     | 68         |
| 3           | 72  | 72  | 72  | 72  | 84  | 84  | 96  | 120 | 126 | 138 | 120 | 96  | 90  | 78  | 84  | 78  | 58         |
| 4           | 90  | 84  | 96  | 108 | 96  | 108 | 114 | 102 | 114 | 108 | 114 | 114 | 72  |     |     |     | 72         |
| 5           | 90  | 84  | 90  | 90  | 90  | 102 | 102 | 96  | 102 | 108 | 114 | 114 | 120 | 108 | 96  | 90  | 87         |
| 6           | 96  | 96  | 102 | 96  | 102 | 120 | 132 | 138 | 126 | 102 | 96  |     |     |     |     |     | 47         |
| 7           | 78  | 84  | 78  | 72  | 78  | 78  | 90  | 90  | 102 | 108 | 108 | 90  | 84  |     |     |     | 64         |
| 8           | 96  | 96  | 102 | 102 | 102 | 114 | 120 | 126 | 132 | 108 | 102 | 96  |     |     |     |     | 46         |
| 9           | 78  | 72  | 72  | 72  | 90  | 102 | 126 | 126 | 114 | 90  | 90  | 72  |     |     |     |     | 42         |
| 10          | 102 | 108 | 114 | 114 | 126 | 126 | 132 | 132 | 126 | 114 | 108 | 108 | 102 | 102 | 102 |     | 39         |
| 11          | 84  | 84  | 96  | 84  | 96  | 102 | 114 | 114 | 114 | 102 | 96  | 96  | 90  |     |     |     | 57         |
| 12          | 102 | 108 | 108 | 102 | 114 | 108 | 108 | 108 | 108 | 108 | 108 | 96  | 80  |     |     |     | 80         |
| 13          | 108 | 114 | 114 | 120 | 132 | 144 | 144 | 150 | 144 | 144 | 126 | 120 | 108 |     |     |     | 40         |
| 14          | 72  | 78  | 78  | 90  | 108 | 108 | 108 | 108 | 90  | 72  | 78  | 84  |     |     |     |     | 42         |
| 15          | 78  | 102 | 96  | 90  | 102 | 108 | 114 | 114 | 114 | 102 | 90  |     |     |     |     |     | 48         |
| 16          | 90  | 90  | 84  | 90  | 84  | 114 | 108 | 108 | 114 | 114 | 108 | 102 | 90  |     |     |     | 60         |
| 17          | 84  | 78  | 78  | 84  | 84  | 84  | 96  | 102 | 120 | 108 | 90  | 84  |     |     |     |     | 46         |
| 18          | 72  | 72  | 72  | 66  | 72  | 66  | 72  | 72  | 78  | 84  | 84  | 90  | 90  | 84  |     |     | 88         |
| 19          | 66  | 72  | 66  | 72  | 72  | 66  | 66  | 78  | 84  | 84  | 96  | 96  | 96  | 84  |     |     | 83         |
| 20          | 90  | 102 | 96  | 96  | 90  | 108 | 114 | 120 | 108 | 104 | 84  |     |     |     |     |     | 38         |
| 21          |     | 96  | 90  | 96  | 114 | 126 |     | 132 |     |     |     |     |     |     |     |     | 30         |
| 22          | 102 | 108 | 114 | 120 | 138 | 138 |     | 126 |     |     |     |     |     |     |     |     | 30         |
| 23          | 90  | 90  | 96  | 102 | 108 | 108 | 102 | 102 | 108 | 114 | 114 | 96  | 96  | 90  | 90  |     | 66         |
| 24          | 90  | 96  | 102 | 108 | 114 | 114 | 108 | 108 | 120 | 114 | 102 | 90  | 90  | 84  |     |     | 59         |
| 25          |     | 90  | 96  | 96  | 96  | 108 | 114 | 114 | 108 |     | 108 | 102 | 84  | 84  |     |     | 63         |
| 26          | 84  | 84  | 96  | 102 | 114 | 120 | 126 | 120 |     | 114 | 102 | 90  | 84  | 78  | 84  |     | 52         |
| 27          | 102 | 96  | 102 | 108 | 96  | 108 | 108 | 114 | 114 | 102 | 96  | 96  |     |     |     |     | 47         |
| 28          | 84  | 90  | 96  | 96  | 102 | 114 | 114 | 120 |     | 114 | 102 | 96  | 90  | 90  | 90  |     | 45         |

and 15 seconds. A total of 86 per cent showed that the activity of the cardiac medullary center was diminished in 15 seconds or less by an increase of the oxygen in the respired air.

Experiments conducted with ten men in the low pressure chamber in which the subject was held at a barometric pressure of 380 mm. (18,000 feet) gave latent periods similar to those obtained with nitrogen. The oxygen was administered in these cases through a rubber tube held in the mouth. It was customary to give oxygen until the pulse had returned to about the normal rate and then to withdraw the oxygen for five minutes, or until the pulse rate had again accelerated, after

which oxygen was given once more. The following cases in which the pulse rate was recorded for intervals of five seconds are typical.

|                     | BEFORE O <sub>2</sub> | O <sub>2</sub> GIVEN               |
|---------------------|-----------------------|------------------------------------|
| B. B. J. { (1)..... | 8, 8, 8, 8, 8, 8      | 8, 8, 7, 7, 7, 7, 7, 6, 7, 6, 7    |
| { (2).....          | 8, 8, 8, 9, 8, 9      | 8, 8, 6, 7, 7, 7, 7, 6, 7, 6, 7    |
| B. R. L. { (1)..... | 10, 9, 9, 9, 9, 9     | 9, 9, 8, 7, 8, 7, 8, 7, 7, 7, 8, 7 |
| { (2).....          | 9, 9, 9, 9, 9, 8      | 9, 9, 8, 8, 7, 7, 8, 7, 7, 7, 7, 7 |

With one exception the ten men reacted to the oxygen administration by a slowing of the pulse rate which was definite within from 5 to 15 seconds. The exceptional case in two trials gave a latent period of 45 seconds.

The total acceleration of the pulse rate in the nitrogen experiments varied between 6 and 72 beats, although it was usually in the neighborhood of 30. The return to normal, when the subject was restored to atmospheric air, was made in from 10 to 15 seconds. It should be noted that in the nitrogen experiments we did not, as a rule, continue the breathing of nitrogen until the subject became unconscious. In the low pressure chamber the return to normal sometimes required two or three minutes of oxygen administration. In some cases the rate became subnormal.

The above reactions of the heart to changes in oxygen tension bring to mind the discussion of the mechanism by which the observed changes are produced. Gasser and Loevenhart (6) have pointed out that the views on the effect of decreased oxidation on the medullary centers may be classified as follows: *a*, a decrease in oxidation cannot cause stimulation; *b*, decreased oxidation may cause stimulation, but only indirectly by increasing the stimulating effect of carbon dioxide, or by causing the formation and accumulation of acid metabolic products; and *c*, decreased oxygen *per se* under proper conditions will stimulate these centers. They support the third view by proving that the responses of the respiratory and the vasoconstrictor centers occur too rapidly to be attributed to the accumulation of acid products. Our own data on the acceleration and retardation of the heart beat give a reaction time that is also too short to lend support to the acid theory or to the idea of accumulation of metabolic products.

If it be admitted that the variations in oxygen in themselves stimulate and depress the medullary heart center, the question of what



constitutes the accelerator mechanism is still unsettled. It is well recognized that the heart may be accelerated in at least four different ways; *a*, by a decrease in vagal tone; *b*, by stimulation of the accelerator center; *c*, by secretion of adrenin; *d*, by an increase in the temperature of the blood (9). That the low oxygen effect is not the result of a decrease in vagal tone seems likely, since the first action of oxygen want is a stimulating one. Mathison (8) found in animals in which the vagi are intact that irregular slowing occurred frequently during asphyxia. This he attributed to the stimulation of the cardio-inhibitory center. Gasser and Loevenhart (6) also found, in animals under low oxygen produced by the use of carbon monoxide or sodium cyanide, that the latent period for the stimulation of the cardio-inhibitory center varied between 15 and 50 seconds. This period, they find, is often obscured by the rapid onset of the depressive effect of low oxygen on this center. The reaction with which we have dealt in our experiments does not find a ready explanation in decreased vagal tone because the response occurred before the cardio-inhibitory center would have been affected by oxygen want.

Since the first effect of low oxygen on the medullary center is stimulating, it is natural to attribute to the accelerator center the increase observed in exposure to low oxygen of rebreathing and low barometric pressure. Nolf and Plumier (10) believe that in the dog they found evidences of increased tonus in the accelerator cardiac nerves during asphyxia. Mathison (11), on the other hand, demonstrated that the acceleration which immediately preceded heart-block during asphyxia was not due to stimulation of the accelerator center. After sectioning the upper part of the spinal cord to remove the influence of the accelerator center, he still obtained acceleration of the heart. That low oxygen may lead to stimulation of the adrenals has been demonstrated by Kellaway (12). He observed a dilatation of the pupils during such an exposure. We have seen a dilatation of the pupils of some of our subjects during the last part of the period while breathing nitrogen. Meek and Eyster (13) have shown that the action of adrenin is twofold. It accelerates the heart by direct stimulation, and inhibits it reflexly through the vagus, the acceleration occurring first. That an increase in temperature is not the cause of the acceleration follows from the briefness of the nitrogen experiments in which the acceleration was evident within 5 to 15 seconds.

We believe that the acceleration which we have reported in this paper and in our study of low pressures and low oxygen percentages,

occurs before the cardio-inhibitory effects observed by Gasser and Loevenhart and by Mathison. It is not the same acceleration that Gasser and Loevenhart found after the depression of the inhibitory center. That it is a result of a stimulating action on the accelerator heart center seems to us the most satisfactory explanation. We have, however, no experimental proof for this explanation.

The beneficial or quieting effect of oxygen on the heart rate has been observed by Benedict and Higgins (14) and by Parkinson (15). In normal individuals at sea level the breathing of oxygen-rich mixtures slowed the rate appreciably. Schneider and Sisco (16), working on Pike's Peak (14,110 feet), administered oxygen to six subjects and observed a reduction in the pulse rate which varied between 7.4 and 28.8 per cent, while in Colorado Springs (6000 feet) the breathing of pure oxygen caused a slowing of from 2.5 to 8.8 per cent.

#### RESPIRATION

The response of the respiratory mechanism to changes in oxygen tension was studied by the same methods and at the same time as the pulse rate changes were under observation. The respiration was recorded by means of a pneumograph and a Mackenzie polygraph. In some cases we have also measured the volume of breathing by means of the Larsen spirometer. From the polygraph tracing the height of the curve of each respiration has been measured to determine a factor which would give relative data on the change in volume of each respiration. The rate of breathing has been determined by noting the time taken for each breath.

A summary of the results obtained with nitrogen is given in table 2. In table 3 the results of six experiments are presented in detail. In the nitrogen experiments we dealt with opposite conditions, a reduction in oxygen tension and an increase in oxygen tension. In the presentation of the pulse rate changes it was shown why the latent period as determined for the effects of decrease in oxygen would be longer than that for the effects of increase in oxygen. In addition to the influence of the depth of breathing, the rate of blood flow may account for the shorter latent period that occurred when the subject was returned to normal atmospheric air. During the early stages of asphyxia, Mathison (11) found in animals that the systolic output per beat gradually increased, reaching a maximum in about 30 seconds. Since the effects of the oxygen changes are brought about through the action on the

TABLE 2

*Effects of breathing nitrogen on respiration. The latent periods for the initial response in both rate and depth, when nitrogen is on and off, are given*

| NUMBER | RATE              |                    |                     |                    |          | HEIGHT            |        |         |                    |          | VOLUME            |                        |         |
|--------|-------------------|--------------------|---------------------|--------------------|----------|-------------------|--------|---------|--------------------|----------|-------------------|------------------------|---------|
|        | On; latent period | Normal per min-ute | Maximum per min-ute | Off; latent period |          | On; latent period | Normal | Maximum | Off; latent period |          | On; latent period | Deciliters per breadth |         |
|        |                   |                    |                     | Begin              | Complete |                   |        |         | Begin              | Complete |                   | Begin                  | Maximum |
|        |                   |                    |                     |                    |          |                   |        |         |                    |          |                   |                        |         |
| sec.   |                   |                    | sec.                | sec.               | sec.     | mm.               | mm.    | sec.    | sec.               | sec.     |                   |                        |         |
| 1      | 32                | 13                 | 27                  | 19                 |          | 19                | 5      | 26      | 10                 |          | 14                | 2.50                   | 19.10   |
| 2      | 60                | 14                 | 23                  |                    |          | 8                 | 4      | 20      | 9                  | 18       | 17                | 2.80                   | 15.70   |
| 3      | 50                | 16                 | 23                  |                    |          | 4                 | 4      | 9       | 4                  |          | 4                 | 5.60                   | 14.80   |
| 4      | 26                | 16                 | 27                  | 7                  |          | 7                 | 4      | 22      | 5                  |          | 7                 | 6.70                   | 26.30   |
| 5      | 8                 | 17                 | 26                  | 5                  |          | 4                 | 5      | 21      | 3                  | 45       | 4                 | 3.90                   | 8.95    |
| 6      | 55                | 16                 | 29                  | 7                  | 20       | 22                | 4      | 16      | 10                 | 31       | 18                | 6.20                   | 17.40   |
| 7      | 20                | 18                 | 24                  | 12                 | 12       | 20                | 6      | 14      | 3                  |          | 23                | 2.24                   | 5.60    |
| 8      | 45                | 17                 | 30                  | 11                 | 25       | 13                | 5      | 22      | 6                  | 27       | 13                | 2.80                   | 8.96    |
| 9      | 33                | 15                 | 17                  | 4                  | 17       | 21                | 14     | 25      | 9                  | 14       | 6                 | 8.40                   | 19.60   |
| 10     | 20                | 14                 | 29                  | 31                 | 31       | 22                | 4      | 8       | 24                 | 31       | 6                 | 3.90                   | 7.84    |
| 11     | 27                | 12                 | 17                  |                    |          | 35                | 5      | 10      |                    |          | 23                | 8.40                   | 13.40   |
| 12     | 31                | 18                 | 30                  | 6                  | 11       | 15                | 4      | 13      | 3                  | 11       | 15                | 4.50                   | 10.80   |
| 13     | 26                | 20                 | 50                  |                    |          | 18                | 4      | 17      | 15                 | 25       | 15                | 2.80                   | 7.80    |
| 14     | 32                | 15                 | 25                  | 11                 | 32       | 12                | 3      | 25      | 5                  | 21       | 4                 | 1.12                   | 12.20   |
| 15     | 41                | 18                 | 21                  |                    |          | 9                 | 4      | 28      | 5                  | 10       | 9                 | 6.72                   | 19.10   |
| 16     | 48                | 18                 | 27                  |                    |          | 7                 | 4      | 30      |                    |          | 7                 | 2.24                   | 22.40   |
| 17     | 61                | 15                 | 23                  | 4                  | 13       | 25                | 3      | 17      | 8                  | 23       | 25                | 2.24                   | 7.84    |
| 18     | 80                | 26                 | 38                  |                    |          | 17                | 3      | 10      | 7                  | 17       |                   |                        |         |
| 19     | 48                | 8                  | 15                  |                    |          | 28                | 9      | 16      | 10                 |          |                   |                        |         |
| 20     | 48                | 9                  | 11                  |                    | 25       | 12                | 6      | 12      |                    |          |                   |                        |         |
| 21     | 25                | 13                 | 37                  | 9                  |          | 20                | 12     | 30      | 9                  |          |                   |                        |         |
| 22     | 10                | 18                 | 25                  | 2                  | 10       | 10                | 7      | 25      | 2                  | 15       |                   |                        |         |
| 23     | 21                | 13                 | 23                  | 7                  |          | 12                | 6      | 13      | 3                  |          |                   |                        |         |
| 24     | 25                | 11                 | 35                  | 3                  |          | 12                | 4      | 15      | 3                  | 20       |                   |                        |         |
| 25     | 34                | 15                 | 20                  | 15                 |          | 12                | 8      | 27      | 11                 | 35       |                   |                        |         |
| 26     | 22                | 17                 | 20                  |                    |          | 22                | 5      | 17      | 5                  | 21       |                   |                        |         |
| 27     | 47                | 18                 | 25                  | 3                  |          | 10                | 5      | 16      | 6                  | 21       |                   |                        |         |
| 28     | 25                | 21                 | 30                  | 8                  | 22       | 10                | 9      | 22      | 3                  | 26       |                   |                        |         |
| 29     | 18                | 23                 | 46                  | 19                 | 37       | 5                 | 7      | 20      | 5                  | 48       |                   |                        |         |
| 30     | 48                | 10                 | 27                  | 7                  | 14       | 13                | 6      | 20      | 6                  | 53       |                   |                        |         |







medullary centers, the shorter latent period at the return to air or oxygen is explained in part by the increased blood flow.

When anoxhemia was produced by breathing nitrogen, the latent period for change in volume of breathing as estimated by the height of the respiratory curve ranged between 4 and 35 seconds. The average was 14.5 seconds. About 32 per cent of all cases have a latent period of 10 seconds or less, while in 29 per cent it was between 10 and 15 seconds. In the determinations of the volume of each breath by the Larsen spirometer the latent period ranged between 4 and 25 seconds, averaging 12.4 seconds. In almost all cases the latent period as estimated by this method was slightly less than that determined by the height of the respiratory curves.

The volume of breathing diminished quickly when the subject was restored to atmospheric air. The latent period as determined by the height of the pneumograph curve ranged between 3 and 34 seconds, averaging 6.9 seconds. A large number, 89.3 per cent, had a latent period of 10 seconds or less. This would indicate that the respiratory center, with respect to the volume of each breath responds to oxygen changes slightly earlier than the cardiac center.

The rate of breathing is increased by anoxhemia when the fall in oxygen has become marked. On administering nitrogen the latent period for the increase in the rate of breathing ranged between 8 and 80 seconds, averaging 35.5 seconds. The normal rate of breathing ranged between 8 and 26 breaths per minute, and at the height of anoxhemia it ranged between 11 and 46 breaths. The rate of breathing decreased more rapidly when the anoxhemia was alleviated than it increased during the withdrawal of oxygen. The latent period for rate on the return to air ranged between 3 and 31 seconds, averaging 9.5 seconds. The respiratory stimulation due to low oxygen with respect to rate and depth passed away completely within from 10 to 53 seconds after returning to atmospheric air.

In a few cases which were kept at 380 mm. in the low pressure chamber we have determined the volume change when oxygen was administered. The per-minute volume of breathing was recorded in these experiments and the data are given in the table. It will be observed that the per-minute volume of breathing was much reduced even during the first minute of oxygen administration. A fall in rate was also present in four cases.

| E. C. S.  |        |      | B. R. L.  |        |      | K. O. N.  |        |      |  |
|-----------|--------|------|-----------|--------|------|-----------|--------|------|--|
| Minutes   | Volume | Rate | Minutes   | Volume | Rate | Minutes   | Volume | Rate |  |
| 16        | 11.4   | 13   | 16        | 12.3   | 11   | 10        | 11.5   | 18   |  |
| 17        | 11.6   |      | 17        | 11.9   |      | 11        | 11.6   |      |  |
| 18        | 11.6   |      | 18        | 13.0   |      | 12        | 11.2   |      |  |
| 19        | 12.8   |      | 19        | 13.3   | 12   | 11.5      | 16     |      |  |
| 20        | 11.0   |      | 20        | 12.7   | 14   | 11.6      |        |      |  |
| 21        | 11.9   | 14   | 21        | 13.9   | 12   | 15        | 11.1   | 18   |  |
| 22        | 11.6   |      | 22        | 15.1   | 16   | 12.2      |        |      |  |
| Oxygen on |        |      | Oxygen on |        |      | 17        | 11.7   |      |  |
| 23        | 8.3    | 12   | 23        | 10.9   | 11   | 18        | 11.9   |      |  |
| 24        | 6.1    |      | 24        | 5.8    |      | 19        | 14.6   |      |  |
| 25        | 10.1   |      | 25        | 6.0    |      | Oxygen on |        |      |  |
| 26        | 4.8    |      | 26        | 6.84   | 9    | 20        | 8.74   | 16   |  |
| 27        | 7.7    |      | 27        | 8.4    | 21   | 7.61      |        |      |  |
| 28        | 6.2    |      | 28        | 4.25   | 22   | 6.72      |        |      |  |
|           |        |      |           |        |      | 23        | 6.95   |      |  |
|           |        |      |           |        |      | 24        | 9.06   |      |  |
|           |        |      |           |        |      | 25        | 8.06   |      |  |
|           |        |      |           |        |      | 17        |        |      |  |
| G. S. M.  |        |      | B. B. J.  |        |      | B. R. L.  |        |      |  |
| Minutes   | Volume | Rate | Minutes   | Volume | Rate | Minutes   | Volume | Rate |  |
| 14        | 11.9   | 12   | 12        | 11.5   |      | 15        | 11.9   |      |  |
| 15        | 9.51   |      | 13        | 11.6   |      | 16        | 14.0   |      |  |
| 16        | 10.1   |      | 14        | 12.5   |      | 17        | 13.1   |      |  |
| 17        | 9.75   |      | 15        | 9.18   |      | 18        | 15.8   |      |  |
| 18        | 12.2   |      | 16        | 13.2   | 23   | 19        | 14.1   |      |  |
| 19        | 18.4   | 12   | 17        | 12.7   | 22   | Oxygen on |        |      |  |
| Oxygen on |        |      | Oxygen on |        |      | 20        | 8.4    |      |  |
| 20        | 14.0   | 11   | 18        | 9.51   | 15   | 21        | 5.60   |      |  |
| 21        | 13.3   |      | 19        | 6.26   |      | 22        | 3.82   |      |  |
| 22        | 11.3   |      | 20        | 8.28   |      | 23        | 3.36   |      |  |
| 23        | 12.3   |      | 21        | 9.06   |      |           |        |      |  |
| 24        | 10.7   |      | 22        | 6.16   |      |           |        |      |  |
| 25        | 10.6   |      | 23        | 7.39   |      |           |        |      |  |

Our data obtained from a study of men indicate that the respiratory center responds to a decrease in oxygen in the same manner and in about the same short time as Gasser and Loevenhart found in animals. The shortness of the latent periods both when oxygen is withdrawn and when it is administered, suggests that the oxygen effects are immediate

and determined only by the time required for the blood to pass from the lungs to the medullary centers. These data appear to lend support to the view that oxygen under certain conditions *per se* determines the condition of activity of the respiratory and other medullary centers.

In this connection the observations of Lindhard (17) are interesting because he found that an excess of oxygen diminished the excitability of the respiratory center. Kaya and Starling (18) made chloralized animals breathe a mixture of nitrogen and oxygen and found that a diminution of the oxygen from 20 to 14 per cent had, as a rule, no effect on the rhythm or depth of respiration, but that oxygen of 8 to 10 per cent increased the amplitude and rhythm of the respiratory movements. The short latent period that we obtained with nitrogen and in our respiration studies in the low pressure chamber and in rebreathing (1) indicates either that a man is more sensitive to changes in oxygen or that chloral alters the excitability of the respiratory center.

The quick responses made by the heart and the respiration to changes in the oxygen tension of the respired air make it appear that the oxygen has a direct influence on the excitability of the medullary centers which control the rate of heart beat and the breathing. Whether oxygen acts indirectly by increasing and decreasing the stimulating effects of carbon dioxide or whether oxygen itself, by the variation in partial pressure or in the rate of oxidation, is a stimulus to the medullary centers, still remains an unsettled question.

#### SUMMARY

1. The cardiac and respiratory medullary centers in man respond quickly to changes in the partial pressure of oxygen. A decrease in oxygen stimulates while an increase in oxygen inhibits the action of these centers.

2. The heart accelerated in from 5 to 55 seconds in response to a decrease in oxygen. In 66 per cent of all cases the acceleration began within 15 seconds or less. Administration of oxygen slowed the heart within from 5 to 30 seconds. In 86 per cent of the cases the retardation began within 15 seconds.

3. Changes in the partial pressure of oxygen in the respired air have a twofold action on the respiration; the rhythm and the depth of breathing may be altered. In a gradual and comparatively slow reduction in oxygen only the depth of breathing was usually increased. With a sudden decrease in oxygen the depth of breathing was first increased and later the rate.



4. The latent period for the increase in the depth of breathing in anoxemia ranged between 4 and 35 seconds, averaging 14.5 seconds. The latent period for the increase in the rate of breathing ranged between 8 and 80 seconds, averaging 35.5 seconds.

5. When the subject was returned to atmospheric air, the latent period for reduction in the volume of breathing varied between 3 and 24 seconds, averaging 6.9 seconds; for the rate of breathing it varied between 3 and 31 seconds, averaging 9.5 seconds.

6. In all subjects at a barometric pressure of 380 mm. (18,000 feet), administration of oxygen reduced the volume of breathing, and in some cases the rate also was decreased.

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# EXPERIMENTAL STUDIES OF THE URETER

## THE CAUSE OF THE URETERAL CONTRACTIONS

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### INTRODUCTION

The first paper on the study of the ureter was devoted chiefly to an investigation of its movements and innervation, making use of ring preparations and the contractions of the ureters *in situ*, but the cause of the ureteral contractions received little attention. A number of experiments in this connection have been reported by other observers, but the conclusions reached do not accord.

Some investigators, such as Mulder, Donders, Mayer, Ludwig, Henderson, Lucas and others, consider distention of the ureter lumen by an accumulation of urine as the main cause of the contractions. On the other hand, Vallentin, Vulpian, Setschenow and others do not regard a certain amount of urine as a necessary condition for the development of spontaneous contractions, since they observed contractions which were produced by other measures, such as by stimulating ganglions of the abdominal and lumbar sympathetics. Although Engelmann obtained an augmentation of ureteral contractions in experimental animals after giving a large amount of fluid, yet he is inclined to consider the distention as neither the cause nor the causal condition of the contractions because first, no contractions were produced even by artificial distention of the ureter lumen; and second, normal movements of the ureter continued even in complete absence of urine secretion. Sokoloff and Luchsinger placed a whole ureter removed from an animal into normal salt solution, connecting both ends with cannulas. In their experiments an increase of the intra-ureteral pressure caused a corresponding acceleration in the rate of contractions, but if the pressure exceeded a certain maximum limit, the contractions ceased. Lewin and Goldschmidt also favor the theory that the ureteral contractions depend entirely upon the urine, which enters into the ureter and distends its lumen. Other clinical

observations in cases of ectopia vesicae by Slanski, Samschin, Greif-Smith and Feodrow throw no light on this question. Protopopow performed a series of experiments on the ureteral contractions under various circulatory and secretory conditions of the kidney, and also after injecting several fluids into the renal pelvis. He reached the conclusion, from these experiments, that the passing of the urine through the ureter lumen is not a necessary condition for spontaneous ureteral contractions, although it has a definite influence upon the contraction rate.

#### METHODS

The experiments were carried out by two methods. Dogs were used, and after anesthesia by morphin and ether, were so firmly fixed on a holder that movement was impossible.

In the first method the left abdominal cavity was opened obliquely from a point which lay at the lower border of the last rib nearly two inches laterally from the linea alba, to the symphysis pubis. This incision ran almost parallel to the course of the left ureter. An additional incision a few inches long was made along the last rib, starting at the upper end of the sagittal incision toward the outer side. The intestines were drawn into the right half of the abdominal cavity, and the abdominal wall was covered with an electric pad to prevent cooling of the viscera. In this way the left ureter together with the left kidney and the bladder could be seen, and the abdominal cavity was opened so widely that the ureter was not influenced by any respiratory or circulatory movements of other parts of the body. Usually the spontaneous peristalsis of the ureter continued for a few minutes only. After opening the bladder a small cannula (outlet cannula) was introduced into the orifice of the left ureter, the urine flow through the cannula being recorded by a drop tambour. A long, thin venous cannula was led to the renal pelvis of the same side through the kidney parenchyma, by means of which various solutions were injected into the ureter lumen (inlet cannula).

Although both ends of the ureter were connected water-tight with two cannulas, the nerves and blood supply which come up to the ureter from the kidney and the bladder were little injured by this method, the ureter remaining in a relatively normal condition except for direct exposure to the air. The left abdominal cavity, thus made almost empty, was heated from a distance by a nitrogen lamp to a temperature of nearly 38°C. To keep the ureter moist, a small amount of Locke's

solution was poured into the abdominal cavity. A point of the ureter was connected to a lever with a small hook, whereby a small section adjacent to this point was suspended in the shape of an inverted V. As a peristaltic contraction passed through this point, this section was stretched in a straighter line, pulling the lever downward, the other end of the lever tracing a curve on a revolving drum. Curves obtained by these methods, therefore, represent contractions of the longitudinal muscles of the ureter. But in the ureter *in situ* both contractions of the longitudinal and the circular muscle layers occur at the same time, so that the curves can be regarded as representing, in time, contractions of the circular muscles. This conclusion is corroborated in the present experiments by the fact that the urine flow was blocked during the peristalsis, which caused a closure of the ureter lumen by the contraction of the circular muscle. By this method the experimental results 1 to 7 were obtained.

In the second method the dog was laparotomized by an incision nearly five inches along a line running laterally and upward from the symphysis pubis on the left side of the abdominal wall. The lower end of the ureter was connected with an outlet cannula in the same manner as in the first method; the kidney and the upper half of the left ureter remained in the natural condition and were protected against exposure to the air, yet movements of the body due to respiration and circulation were occasionally transmitted to the lever, which to a great extent spoiled the true curves of the ureteral contractions. A point of the ureter also was suspended to the lever and several fluids were injected into the femoral vein. By this method experimental result 8 was obtained.

#### EXPERIMENTAL RESULTS

1. Injection of 0.9 per cent salt solution into the renal pelvis: With a low pressure of injection the ureter, which previously had no spontaneous contractions, began to register a few contractions per minute. Fluid from the outlet cannula disappeared during the course of a peristaltic wave through the length of the ureter. The rate of contractions then increased approximately proportional to the raising of the pressure to a certain limit. But with a pressure higher than that limit no contractions were observed, the solution flowing more rapidly and uninterruptedly.

2. Injection of 1 per cent urea solution into the renal pelvis: This also caused a definite increase in ureteral activity. The rate of the



contractions, however, was greater than that caused by injecting salt solution at the same pressure, the number of contractions being at least one and a half times as many in comparison. Occasionally tonic contractions occurred and the outflow of fluid ceased for a rather long interval of time. By injecting salt solution after urea, the ureter showed less irritation, there being noticed a series of separated contractions, fewer in number, and no tonic contracture.

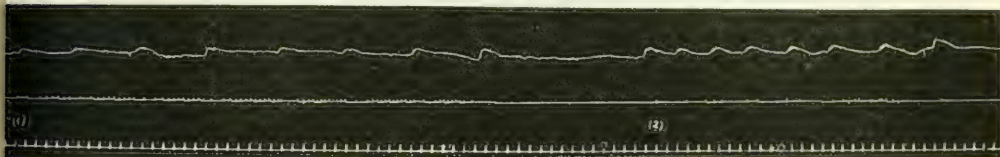


Fig. 1. Portion of graphic tracing obtained in experiment of July 7, 1919. Upper record, contractions of the ureter. Middle record, urine flow. Lower record, base line and time record in minutes. 1, injection of salt solution. 2, injection of urea solution (1 per cent).

3. Injection of a saturated uric acid solution into the ureter lumen: This solution produced an acceleration in the rate of contractions almost equal to that caused by the urea solution. But tonic contractures were observed, greater in number and longer in duration than after injection of the urea solution.

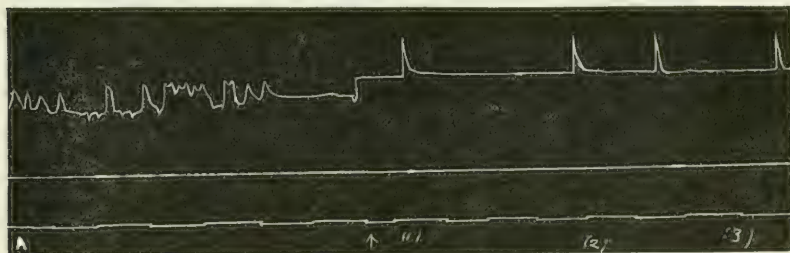


Fig. 2. Portion of graphic record obtained in experiment of July 3, 1919. Upper record, ureteral contractions. Middle record, urine flow. Lower record time in minutes. A, injection of uric acid solution (saturated); ↑, death of animal; 1, 2, 3, injections of salt, uric acid and urea solutions respectively.

4. Injection of urine of the respective animals into the renal pelvis: The urine exerted a distinctly favorable influence upon ureteral activity, contractions being more frequent than after salt solution. They presented, however, less tendency to tonicity. Salt solution after urine lessened the contractions.

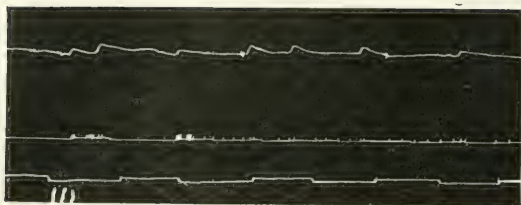


Fig. 3. Portion of graphic record obtained in experiment of June 23, 1919. Upper record, ureteral contractions. Middle record, urine flow. Lower record, time in minutes. 1, injection of urine into the renal pelvis.

5. Injection of glycerin-water mixture and cod liver oil into the ureter lumen: For the purpose of deciding whether the viscosity of fluids which pass through the ureter lumen has any effect upon the movements of the ureter, glycerin in various concentrations and cod liver oil were injected into the renal pelvis. A weak solution of glycerin (5 per cent) had the same effect as salt solution. An increasing concentration gave no distinct acceleration of the contractions, either in rate or in force. The injection of cod liver oil did not produce the augmentation caused by urea or uric acid solutions.

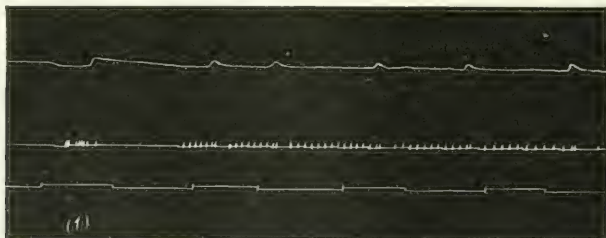


Fig. 4. Portion of graphic record obtained in experiment of June 23, 1919. Upper record, ureteral movements. Middle record, urine flow. Lower record, time in minutes. 1, injection of glycerin water mixture.

6. Injection of drugs into the ureter lumen: In the previous paper the effects of adrenalin, physostigmin and atropin upon ring preparations were described. These drugs were employed also in the present experiments, and their effects on the ureter in situ were the same as upon the excised ureter. Adrenalin caused marked rapid movements with no pause between them, but no increase in tonus. Physostigmin also produced an augmentation in rate of contractions together with a

slight increase in tonus. Atropin exhibited a gradual decrease in force to a final disappearance of contractions, though it seemed to affect the rate of contraction very slightly.

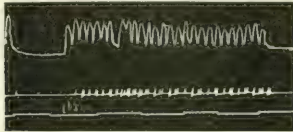


Fig. 5a.

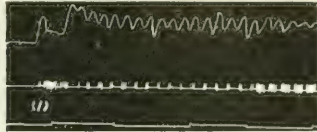


Fig. 5b

Fig. 5, a. Portion of graphic record obtained in experiment of July 1, 1919. Upper record, ureteral contractions. Middle record, urine flow. Lower record, time in minutes. 1, adrenalin injection (1 to 1,000,000).

Fig. 5, b. Portion of graphic record obtained in experiment of July 1, 1919. Upper record, ureteral movements. Middle record, urine flow. Lower record, time in minutes. 1, injection of physostigmin (1 to 200,000).

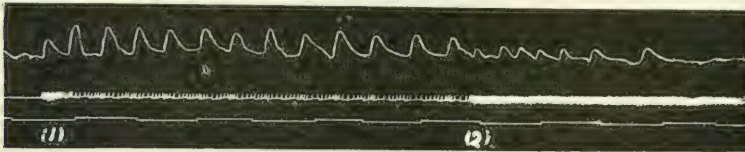


Fig. 5, c. Portion of graphic record obtained in experiment of July 1, 1919. Upper tracing, ureteral contractions. Middle record, urine flow. Lower record, time in minutes. 1, atropin injection (1 to 400,000). 2, injection of the drug with a higher pressure.

7. Injection of sand-water mixture into the ureteral lumen: Even with a very low pressure, at which salt solution produced only a few contractions per minute, the ureter contracted in a tonic manner, its tone increasing markedly and contractions following successively with no interruption. A larger amount of the mixture caused a strong con-

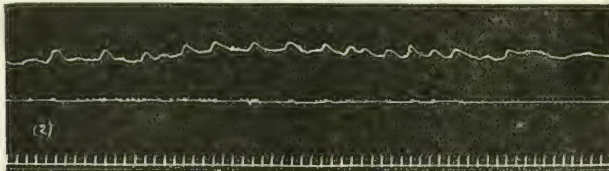


Fig. 6. Portion of graphic record obtained in experiment of July 7, 1919. Upper record, ureteral movements. Middle record, urine flow. Lower record, time in minutes. 2, injection of sand-water mixture.



traction of the ureter, only a few drops of the fluid being expelled from the outlet cannula. The circular muscle layer seemed to be especially stimulated, so that the ureter was put in a condition of vermicular movements, which continued to travel up and down its different sections.

8. Injection of salt solution and urea intravenously: Five to ten minutes after injecting 50 to 100 cc. of 0.9 per cent salt solution into the femoral vein, drops of urine appeared at a certain rate, sometimes accompanied by a number of distinct contractions, again, the ureter remaining perfectly quiescent. The urea injection (1.0 gm. in 10 cc. solution) presented nearly the same effect, contractions, however, being slightly more in number and stronger in force.

From these experiments definite evidence is obtained in the first place, that the distention of the ureter lumen produces contractions. This was again proved in an experiment in which the outlet cannula was clamped off to prevent the flow of urine for a while, so that the intra-ureteral pressure was very much heightened. In response to this increase of pressure, the ureter began to contract after a short period of time and showed an increase of activity, the tonus and amplitude being heightened with each contraction until the outlet cannula was again opened. This fact indicates clearly that the distention of the ureter lumen by an accumulation of urine secretion may be an important cause of ureteral contractions.

In the second place, it is probable that the urine may exert a chemical influence upon the ureteral movements. It is for the purpose of studying this point that urea and uric acid were injected into the renal pelvis through the inlet cannula. As stated above it was found that urea and uric acid solutions caused a more vigorous activity of the ureter than a neutral solution (salt solution) under the same distention of the ureter lumen. The ureter also contracted more frequently when the normal urine of the animals was injected. The peristaltic contractions of the ureter, therefore, depend not only upon the mechanical distention of the ureter lumen, but also, to a certain extent, upon the composition of the urine.

It is a noteworthy feature that these agents, when applied only on the mucous surface of the ureter, affect its movements. This fact implies that the contractions of the muscle layer must be brought about in a reflex manner, stimulus of the sensory nerves of the mucosa being transmitted by the ganglion cells to the motor nerves of the muscles, which then are thrown into a condition of contractions. Occa-





Fig. 7. Portion of graphic record obtained in experiment of June 9, 1919. Upper record, ureteral movements. Middle record, urine flow. *I*, injection of 50 cc. of salt solution intravenously. *A*, opening of outlet cannula.

sion was had in one of the experiments to corroborate the truth of this conclusion: It was observed that after the death of the animal contractions could be obtained by irrigating the ureter with the different solutions described, but under these conditions the contractions were the same in rate and form in spite of the injection of different fluids such as salt, urea, uric acid solutions and urine. The experiment indicates that in the absence of certain nervous actions, injection of various fluids effects merely a mechanical distention of the surviving muscle layer and produces its contractions in consequence of this factor alone. Further, the vigorous tonic contracture caused by injecting sand-water mixture, which may be regarded as a result of stimulation by solid bodies acting on the mucous surface, may be considered as definite proof that there is a reflex mechanism in the development of the ureteral peristalsis.

Under normal conditions distention should be regarded as acting in part directly on the muscle and in part as causing a reflex contraction. This conclusion is indicated at least by the fact that in some of the experiments the ureteral contractions after the death of animals were strikingly different in rate and shape from those obtained in life, the former being quicker in duration and slower in rate than the latter. This difference depends probably upon the absence of the nervous factor. By distention the sensory nerves of the ureteral wall are probably stimulated and thus have a reflex effect upon the muscular layer, initiating or modifying the resulting contraction.

#### DISCUSSION

As generally accepted, the ureter may be considered as having the power of independent contraction to a relatively great extent. When the muscle layer is kept in a sufficiently tonic condition, its contractility is manifested in visible contractions. This tonic condition of the ureter sufficient to support contraction is brought about by various mechanisms, such as nervous control, either by direct stimulation of the motor nerves (as the author has shown in the first paper) or by way of a local reflex, by mechanical stretch of the muscle fibers themselves, by direct chemical effect upon the muscle tissue (as excised ureter in Locke's solution), etc. In the natural position of the organ the nervous networks of its wall probably keep it in such a tonic condition that an additional slight increase of the tone—for instance, by distention of the lumen or by chemical influence of urine—can cause its contractions.

After opening the abdominal cavity its tone is very much lessened, probably because of different circulatory conditions of the ureter, loss of temperature and other circumstances, so that the contractions cease after a short interval of time. If the tone, however, is restored to a sufficient degree to cause contractions either mechanically by great distention of the lumen, which effects a stretching of the muscle fibers, or chemically by drugs, which act reflexly, the ureter begins to contract again and goes on while these effects continue. On the other hand, if the motor nerve is stimulated adequately to keep the ureter in sufficient tonus, its contractions may take place even in an absence of other factors, as was practically observed by some authors and the writer.

The ureteral movements, therefore, are produced by various mechanisms,—such as stimulation of the motor nerves, distention of the lumen, chemical effect of the urine,—each of which may, alone, cause contractions of the ureter or which may coöperate with one another.

#### CONCLUSIONS

1. Distention of the ureter lumen causes the development of contractions, which increase to a certain limit with an increase in pressure.
2. The chemical composition of the kidney secretion may also effect the development of ureteral contractions, by means of a reflex action.
3. The viscosity of the fluids which pass through the ureter lumen affects the contractions only to the slightest degree. Solid bodies, such as a calculus, however, produce vigorous tonic contractions.
4. The peristalsis of the ureter should be referred not to one, but to several factors, which act mainly in coöperation, but which vary in value under various conditions.

## THE EFFECTS OF INCREASING THE INTRACRANIAL PRESSURE IN RABBITS

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In a series of "heat puncture" experiments with rabbits (1) the results were at times complicated by convulsions and death. It was thought possible that the symptoms and death in these cases might be due to an increase in the intracranial pressure. To throw light on the subject the general question of the effects produced by increasing the intracranial pressure is being studied. The present paper gives a preliminary report of the investigation.

A number of early workers report the results of experimentally increasing the pressure in the cranial cavity by cerebral compression in animals; and also of clinical cases of traumatic cerebral lesions. The latter, in fact, comprise the bulk of the evidence. Horsley (2) was interested in the subject from a clinical point of view. Using dogs, he increased the intracranial pressure by means of a rubber bag distended with mercury and found that a definite increase always resulted in death, which he interpreted to be due to an arrest of the respiratory movements. Release of the pressure and artificial respiration were effective in the recovery of the animals. Cushing (3) obtained similar results but ascribed death to a paralysis of the vasomotor center in the medulla subsequent to a prolonged stimulation of the center causing a marked rise (250 mm. Hg.) in blood pressure.

More recently Dixon and Halliburton (4) using a method somewhat similar to that of Horsley (2) obtained detailed results of moderate and great changes in the cerebrospinal pressure in dogs. They increased the pressure by forcing Ringer's solution into the craniospinal cavity through a cannula in the subcerebellar cisterna. Moderate degrees of compression, as 80 mm. of mercury, applied for a few seconds produced effects clearly due to stimulation of the principal bulbar centers. The heart was slowed by vagus stimulation, the blood pressure raised by stimulation of the vasomotor center, and the respiration rate,



sometimes initially increased, but always finally diminished. When the pressure was above the arterial pressure (300 to 400 mm. of mercury) respiration ceased in a few seconds; while blood pressure rose rapidly if artificial respiration were used until the vagus and finally the vasomotor center became paralyzed. On removal of the compression the centers recovered in the reverse order. Death always followed pressures of 80 mm. of mercury or more applied for only a few seconds unless artificial respiration was resorted to.

Cannon (5) obtained somewhat similar results with cats. Increasing the intracranial pressure by concussion caused cessation of respiration. Cannon (5) also, in reporting clinical findings in cases of brain lesions, states that there is generally evidence of a rupture of the intracranial blood vessels and changes in the osmotic condition of the brain substance causing a rise in intracranial pressure. The symptoms are unconsciousness, coma, clonic spasms, labored breathing, slow heart beat, rise in body temperature and death unless decompression is resorted to; all analogous to those produced by experimentally increasing intracranial pressure in animals. The clinical symptoms are reported in many other cases by numerous observers.

There are no data in the literature on the effect of increasing the intracranial pressure in rabbits. The evidence reported in the present paper, although incomplete as yet, is being extended.

The symptoms preceding death in the fatal cases of "heat puncture" were so markedly similar to those produced by experimentally increased intracranial pressure and to clinical findings, that an attempt was made to prove that the cause of death was the same in all these cases. In order to do this the conditions during "heat punctures" were controlled so as to insure a pressure in the cranial cavity comparable to that produced artificially or by brain lesions. The cylinder used in puncture experiments to hold the puncture needle was screwed firmly into the trephine opening in the skull and the hole closed tightly by the puncture needle or other means so that any pressure due to intracranial hemorrhage or changes in the brain substance would result in an actual increase in the pressure in the cranial cavity. Because of the impossibility of passing a needle through the dura, cortex, and into the corpus striatum without rupturing small blood vessels, autopsies almost invariably showed some degree of hemorrhage which would be sufficient to raise the pressure to some extent.

Artificially produced pressure in the cranial cavity was produced by means of a metal cylinder screwed firmly into the trephine opening

in the skull, the opening having been made without injury to the dura. In the cylinder was securely fastened a metal tube 4 mm. in diameter and 3 or 4 cm. in length, to which was fastened a rubber tube connected with a bulb by means of which air could be forced into the opening in the skull, increasing the pressure within the cavity. The tube and bulb were both connected with a mercury manometer which recorded the pressure used. The method was varied in certain experiments by using a rubber bag inserted through the cylinder into the trephine opening in the skull. In some cases the bag was distended with 5 to 10 mm. of mercury and the opening closed; in others the mercury was run into the bag from a burette, so that the pressure applied could be accurately gauged by the height of the column of mercury.

The symptoms produced were the same with each method. A pressure of 30 to 40 mm. of mercury (272 to 408 mm. of water) or more applied one to three minutes caused, at first, increase in the rate of respiration followed by a decrease, a slowing of the heart rate, vasoconstriction, dilatation of the pupils, short clonic spasms probably due to asphyxiation and, finally, a cessation of respiration. If, at this stage, the pressure was immediately released, the respiration was again resumed and the other symptoms passed off. Application of the pressure again for only a few seconds caused a repetition of the above, release again bringing recovery. This may be repeated a number of times, but if the pressure was applied for more than a few seconds after respiration ceased, recovery was impossible without the use of artificial respiration.

Twelve experiments were performed on rabbits, using fatal pressures 20 to 30 mm. of mercury, (272 to 408 mm. of water) with similar symptoms and death occurring in every case in which the pressure was applied for a sufficient length of time, generally from one to three minutes.

Twenty-three experiments on rabbits showed that a moderate increase, 15 mm. of mercury or less, (200 mm. of water) in intracranial pressure produces less marked effects, increase in the rate of respiration and vasoconstriction with a subsequent rise in body temperature. This phase of the subject is being further investigated.

The symptoms following the "heat punctures" in which care was taken to have the puncture hole securely closed so that an increase in the pressure in the brain case was possible, were in general comparable to those just stated for artificially increased pressure. The pressure symptoms, in most cases, occurred two or three hours after the "puncture" operation. There was an increased rate of respiration followed by slow, labored breathing, slow heart beat, dilatation of pupils, vaso-

constriction, and generally a rise in body temperature. Clonic convulsions of short duration soon came on, followed by a fall in temperature just prior to death. In some cases the pressure effects occurred earlier (within one-half to one hour); in others later, five to ten or even twelve hours after the operation. Death could be prevented by a decompression operation; that is, by making a small opening in the skull or by removing the puncture needle or cylinder thus preventing the increase in pressure within the brain case. The symptoms occurred only in those cases in which no opening was left in the skull; and, of the thirty cases of puncture in which there was definitely no opening left, twenty-eight showed pressure symptoms followed by death. There were also twenty-four other cases of puncture with fatal pressure symptoms which occurred early in the series and of which no record was kept in regard to the opening in the skull. None of the twenty punctures with the cylinder or needle definitely removed, or with an extra opening in the skull, showed any of the effects of increased pressure in the brain cavity except possibly a rise in body temperature.

Since fatality and the preceding effects are noted only when the brain cavity is kept air-tight by eliminating any opening in the skull, thereby allowing the possibility of an increased pressure in the cranium, in case of hemorrhage or increased brain volume they would seem not to be due directly to the puncture operation but rather to an increased intracranial pressure. Also, the symptoms accompanying death are so like those induced by artificially increasing the pressure and by brain lesions that this conclusion seems justified.

The explanation of the cause of the fatal symptoms is the same as that offered by Dixon and Halliburton (4). The increased pressure at first acts as a stimulus to the bulbar centers and finally, when continued, causes paralysis of the same centers, that of respiration being the first to be affected. The stimulation effect is evidenced by the early increase in the rate of respiration by stimulation of the respiratory center, by the slowing of the heart rate through the cardio-inhibitory center, by vasoconstriction through the vasomotor center, and by dilatation of the pupils through the cervical sympathetic nerve. Over-stimulation, as would be expected, brings about a paralysis of the same centers and a cessation of their functioning.

The fatal pressure recorded for rabbits, 20 mm. of mercury (272 mm. of water) is considerably lower than that found by Dixon and Halliburton (4) for dogs. The difference is probably due to the higher blood pressure in dogs, and to the difference in the method used in raising the intracranial pressure, and place of application of the pressure.

## SUMMARY

Increasing the intracranial pressure in rabbits, 20 mm. of mercury (272 mm. of water) or more, results in accelerated respiratory movements followed by their cessation, slow heart beat, vasoconstriction, dilatation of the pupils, spasms of asphyxiation, and finally death within one to three minutes unless artificial respiration is used or the pressure is released.

Moderate degrees of pressure, 15 mm. of mercury (200 mm. of water) or less cause increase in the rate of heart beat, vasoconstriction and a rise in body temperature.

The pressure symptoms and death following "heat puncture" operations were found only when no opening was left in the cranium; and, by comparison with the symptoms attending artificially increased intracranial pressure and clinical cases of brain lesions, seem to be due to the same cause.

The cause of death, and the preceding symptoms, when the intracranial pressure is raised appears to be stimulation followed by paralysis of the principal bulbar centers.

The author wishes to express her gratitude to Prof. S. S. Maxwell for his advice in this investigation.

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# ON THE DISTRIBUTION OF THE NON-PROTEIN NITROGEN IN CASES OF ANAPHYLAXIS AND PEPTONE POISONING

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Since Richet (1) in 1902 first studied the problem of anaphylaxis systematically, a number of investigators have worked on similar lines and advanced various theories to explain why the lesion and symptoms of anaphylaxis take place after protein has been injected into an animal previously sensitized with the homologous serum.

I do not wish to give a detailed description, only stating that there exist two theories of anaphylaxis, namely, the humoral or chemical theory and the cellular one. The former, as many authors (2), especially Friedberger (3) have pointed out, ascribed the source of anaphylaxis to the protein poison produced from the products of a reaction between free or circulating anaphylactic-antibody and antigen by the action of a complement. Although there is some diversity of opinion as to detail, i.e., whether the protein that is broken down is the protein injected, as Vaughan and Friedberger (2) claimed, or the protein of the sensitized animal itself, as was reported by Friedemann (4), Pfeiffer and Mita (5), Jobling and Peterson (6), yet they are fundamentally in agreement in that they believe that a protein poison is responsible for the anaphylaxis. This theory of anaphylaxis is supported by a mass of experimental data especially in vitro, bearing on the production of the protein poison. According to the second theory it is believed that the antibody is within the cells and the antigen-antibody reaction occurs in this place rather than in the blood stream.

Weil (2) who emphasized the cellular theory most strongly held that anaphylactic shock has no relation to the chemical poison but rather is a transitory shock as the result of a reaction between the cellular antibody and the circulating antigen. From the fact that there was no evidence of the protein poison in his transfusion experiments, he concludes that the phenomenon is attributable to the cellular reaction involving the hepatic parenchyma (7).

The question as to which theory is more plausible, the humoral or the cellular one, must be determined by further studies.

Zunz and György (8), who examined the alternation of the blood nitrogens, stated that there is a definite increase in amino-acids during acute shock in dogs. Jobling and Peterson (6) found that the acute shock is accompanied by an increase in non-coagulable nitrogen of the blood in addition to that of the amino-acids.

Recently Whipple and Van Slyke (9) have reported their detailed investigation on the influence of the proteose intoxication upon the nitrogenous products of the blood, pointing out that the acute shock following an injection of a toxic proteose is usually associated with a large increase in the non-protein nitrogen of the blood, chiefly in the blood urea nitrogen, and also with small increases in the amino and peptiden nitrogens. Keeping these results in mind my investigation was carried out to determine whether this would be the case also in anaphylaxis as in proteose intoxication.

#### METHOD

Guinea pigs weighing 200 to 400 grams were employed in all experiments. In order to avoid the possible influence of diet upon the nitrogenous constituents of the blood, the pigs received no food for about twenty-four hours previous to being bled. The animals were exsanguinated in all cases by opening the carotid artery and the blood was quickly defibrinated with a stirring rod. The blood thus obtained from three or four pigs was thoroughly mixed and urea nitrogen, total non-protein nitrogen and amino-acid nitrogen were determined.

1. *Urea nitrogen.* Van Slyke and Cullen's method (10) was employed, using methyl-alcohol as a substitute for octhyl-alcohol.

2. *Total non-protein nitrogen.* After removal of the protein by the heat-caolin method (sometimes heat-trichloroacetic acid method was used) the micro-Kjeldahl method of Folin and Farmer (12) was employed, the ammonia being titrated with 0.02N acid and 0.01N alkali, using methyl-red as an indicator.

3. *Amino nitrogen* was estimated by Van Slyke's method, the protein being removed by Okada's heat-caolin method (12).

The guinea pigs were divided into three groups. In the first, pigs which had not been submitted to any experimental procedure were used as controls. In the second, 2 cc. of 10 per cent peptone (Witte) solution per 100 gram of body weight were administrated intraperitoneally to

TABLE 1  
*Contents of blood nitrogen of normal guinea pigs*

| EXPERIMENT<br>NUMBER | GUINEA PIG   | TOTAL NON-<br>PROTEIN NITRO-<br>GEN | NON-PROTEIN NITROGEN PER 100 CC. OF BLOOD AS |             |                |
|----------------------|--------------|-------------------------------------|--|-------------|----------------|
|                      |              |                                     | Urea   | Non-urea    | Amino nitrogen |
|                      | <i>grams</i> | <i>mgm.</i>                         | <i>mgm.</i>                                  | <i>mgm.</i> | <i>mgm.</i>    |
| I                    | 1. 320       | 57.6                                | 36.4   | 21.2        |                |
|                      | 2. 365       |                                     |  |             |                |
|                      | 3. 284       |                                     |  |             |                |
| II                   | 4. 396       | 53.1                                | 33.2   | 19.9        |                |
|                      | 5. 252       |                                     |  |             |                |
|                      | 6. 374       |                                     |  |             |                |
| III                  | 7. 380       | 64.5                                | 38.4   | 26.2        | 5.7            |
|                      | 8. 410       |                                     |  |             |                |
|                      | 9. 342       |                                     |  |             |                |
| IV                   | 10. 274      | 55.8                                | 29.2   | 26.6        | 5.9            |
|                      | 11. 360      |                                     |  |             |                |
|                      | 12. 255      |                                     |  |             |                |
| V                    | 13. 294      | 59.8                                | 37.1   | 22.7        | 5.6            |
|                      | 14. 290      |                                     |  |             |                |
|                      | 15. 320      |                                     |  |             |                |
| VI                   | 16. 353      | 55.0                                | 28.9   | 25.1        |                |
|                      | 17. 297      |                                     |  |             |                |
|                      | 18. 377      |                                     |  |             |                |
| VII                  | 19. 294      | 57.3                                | 33.2   | 24.1        | 5.1            |
|                      | 20. 360      |                                     |  |             |                |
|                      | 21. 256      |                                     |  |             |                |
| VIII                 | 22. 405      | 56.3                                | 34.1   | 22.2        | 5.4            |
|                      | 23. 335      |                                     |  |             |                |
|                      | 24. 200      |                                     |  |             |                |
| IX                   | 25. 345      | 59.9                                | 36.7   | 23.2        | 5.1            |
|                      | 26. 376      |                                     |  |             |                |
|                      | 27. 238      |                                     |  |             |                |
| X                    | 28. 198      | 56.0                                | 31.3   | 24.7        | 4.9            |
|                      | 29. 307      |                                     |  |             |                |
| Average .....        |              | 57.5                                | 33.9   | 23.6        | 5.4            |

TABLE 2  
*Blood nitrogen in peptone poisoning*

| EXPERIMENT<br>NUMBER | GUINEA PIG   | RESULTS           | TIME<br>AFTER<br>INJECTION | TOTAL<br>NON-<br>PRO-<br>TEIN<br>NITRO-<br>GEN | NON-PROTEIN NITROGEN<br>PER 100 CC. BLOOD AS |              |                        |
|----------------------|--------------|-------------------|----------------------------|--|--|--------------|------------------------|
|                      |              |                   |                            |  | Urea   | Non-<br>urea | Amino<br>nitro-<br>gen |
|                      | <i>grams</i> |                   | <i>hours</i>               | <i>mgm.</i>                                    | <i>mgm.</i>                                  | <i>mgm.</i>  | <i>mgm.</i>            |
| I                    | 1. 400       | Moderate symptoms | 6                          | 82.3   | 53.3   | 29.0         | 6.8                    |
|                      | 2. 320       | Moderate symptoms |                            |  |  |              |                        |
|                      | 3. 250       | Severe symptoms   |                            |  |  |              |                        |
| II                   | 4. 300       | Moderate symptoms | 4                          | 71.4   | 44.3   | 27.1         | 6.0                    |
|                      | 5. 360       | Moderate symptoms |                            |  |  |              |                        |
|                      | 6. 352       | Moderate symptoms |                            |  |  |              |                        |
| III                  | 7. 360       | Severe symptoms   | 6                          | 77.1   | 48.7   | 28.4         | 6.7                    |
|                      | 8. 286       | Moderate symptoms |                            |  |  |              |                        |
|                      | 9. 254       | Moderate symptoms |                            |  |  |              |                        |
| IV                   | 10. 304      | Moderate symptoms | 5                          | 89.6   | 52.8   | 36.8         | 9.3                    |
|                      | 11. 332      | Severe symptoms   |                            |  |  |              |                        |
|                      | 12. 264      | Died in 3 hours   |                            |  |  |              |                        |
|                      | 13. 300      | Moderate symptoms |                            |  |  |              |                        |
| V                    | 14. 316      | Moderate symptoms | 5                          | 74.2   | 46.2   | 28.0         | 7.8                    |
|                      | 15. 352      | Moderate symptoms |                            |  |  |              |                        |
|                      | 16. 360      | Mild symptoms     |                            |  |  |              |                        |
|                      | 17. 320      | Moderate symptoms |                            |  |  |              |                        |
| VI                   | 18. 240      | Severe symptoms   | 6                          | 85.4   | 52.4   | 33.0         | 6.2                    |
|                      | 19. 280      | Moderate symptoms |                            |  |  |              |                        |
|                      | 20. 312      | Moderate symptoms |                            |  |  |              |                        |
|                      | 21. 268      | Moderate symptoms |                            |  |  |              |                        |
| VII                  | 22. 315      | Moderate symptoms | 4½                         | 84.6   | 53.0   | 29.8         | 7.0                    |
|                      | 23. 280      | Died in 2 hours   |                            |  |  |              |                        |
|                      | 24. 340      | Severe symptoms   |                            |  |  |              |                        |
| VIII                 | 25. 200      | Severe symptoms   | 6                          | 79.9   | 46.7   | 33.2         | 7.1                    |
|                      | 26. 226      | Severe symptoms   |                            |  |  |              |                        |
|                      | 27. 200      | Moderate symptoms |                            |  |  |              |                        |
|                      | 28. 190      | Severe symptoms   |                            |  |  |              |                        |
| IX                   | 29. 200      | Severe symptoms   | 4½                         | 73.5   | 42.9   | 30.6         |                        |
|                      | 30. 216      | Died in one hour  |                            |  |  |              |                        |
|                      | 31. 205      | Moderate symptoms |                            |  |  |              |                        |
|                      | 32. 240      | Severe symptoms   |                            |  |  |              |                        |
| Average .....        |              |                   |                            | 79.8   | 48.9   | 30.7         | 7.1                    |



TABLE 3  
*Blood nitrogen in anaphylactic shock*

| EXPERIMENT<br>NUMBER | GUINEA PIG   | RESULTS           | TIME<br>AFTER<br>INJEC-<br>TION | TOTAL<br>NON-<br>PRO-<br>TEIN<br>NITRO-<br>GEN | NON-PROTEIN NITROGEN<br>PER 100 CC. BLOOD AS |              |                         |
|----------------------|--------------|-------------------|---------------------------------|--|--|--------------|-------------------------|
|                      |              |                   |                                 |  | Urea   | Non-<br>urea | Amino-<br>nitro-<br>gen |
|                      | <i>grams</i> |                   | <i>hours</i>                    | <i>mgm.</i>                                    | <i>mgm.</i>                                  | <i>mgm.</i>  | <i>mgm.</i>             |
| I                    | 1. 264       | Severe symptoms   | 6                               | 101.2  | 67.7   | 33.5         | 6.7                     |
|                      | 2. 272       | Mild symptoms     |                                 |  |  |              |                         |
|                      | 3. 268       | Severe symptoms   |                                 |  |  |              |                         |
|                      | 4. 212       | Severe symptoms   |                                 |  |  |              |                         |
| II                   | 5. 252       | Severe symptoms   | 6                               | 85.7   | 55.6   | 30.1         | 7.3                     |
|                      | 6. 220       | Moderate symptoms |                                 |  |  |              |                         |
|                      | 7. 212       | Moderate symptoms |                                 |  |  |              |                         |
|                      | 8. 204       | Severe symptoms   |                                 |  |  |              |                         |
| III                  | 9. 340       | Severe symptoms   | 5                               | 106.5  | 74.3   | 32.2         | 7.8                     |
|                      | 10. 288      | Severe symptoms   |                                 |  |  |              |                         |
|                      | 11. 312      | Severe symptoms   |                                 |  |  |              |                         |
|                      | 12. 288      | Severe symptoms   |                                 |  |  |              |                         |
| IV                   | 13. 384      | Mild symptoms     | 5½                              | 79.2   | 48.7   | 30.5         | 6.9                     |
|                      | 14. 272      | Mild symptoms     |                                 |  |  |              |                         |
|                      | 15. 394      | Severe symptoms   |                                 |  |  |              |                         |
| V                    | 16. 196      | Died in 2 hours   | 3                               | 82.7   | 53.5   | 29.2         |                         |
|                      | 17. 204      | Died in 3 hours   |                                 |  |  |              |                         |
|                      | 18. 200      | Severe symptoms   |                                 |  |  |              |                         |
| VI                   | 19. 392      | Moderate symptoms | 6                               | 69.3   | 40.6   | 28.7         | 7.0                     |
|                      | 20. 396      | Moderate symptoms |                                 |  |  |              |                         |
|                      | 21. 380      | Severe symptoms   |                                 |  |  |              |                         |
| VII                  | 22. 204      | Died in 2 hours   | 5                               | 77.5   | 45.3   | 32.2         |                         |
|                      | 23. 200      | Died in 1 hour    |                                 |  |  |              |                         |
|                      | 24. 225      | Severe symptoms   |                                 |  |  |              |                         |
| VIII                 | 25. 340      | Severe symptoms   | 5                               | 99.5   | 63.3   | 36.2         | 9.2                     |
|                      | 26. 280      | Severe symptoms   |                                 |  |  |              |                         |
|                      | 27. 310      | Moderate symptoms |                                 |  |  |              |                         |
|                      | 28. 326      | Severe symptoms   |                                 |  |  |              |                         |
| IX                   | 29. 376      | Moderate symptoms | 6                               | 70.1   | 42.0   | 28.1         | 6.9                     |
|                      | 30. 256      | Severe symptoms   |                                 |  |  |              |                         |
|                      | 31. 250      | Severe symptoms   |                                 |  |  |              |                         |
| Average .....        |              |                   |                                 | 85.4   | 54.6   | 31.2         | 7.4                     |

each pig. In the third group, the animals were sensitized with 0.02 cc. serum and after an interval of 2 or 3 weeks, 1 cc. of the same serum as in the case of group 2, per 100 gram body weight, was injected intraperitoneally. The results are shown in tables 1, 2 and 3.

It is seen from table 2 that the peptone intoxication is accompanied by a marked increase in the non-protein nitrogen of the blood. The urea nitrogen is materially increased. The other non-protein nitrogenous constituents—in our cases non-urea and amino nitrogen—have also shown more or less increases. These results may be in accord with those of experiments with dogs performed by Whipple and Van Slyke who concluded that this increase in non-protein nitrogen is a result of an abnormally rapid tissue autodigestion caused by the action of a toxin (9).

As to anaphylaxis, as indicated in table 3, it has the same influence upon the non-protein partition of the blood as peptone poisoning or rather it appears to be more intense. From these results it appears probable that in anaphylaxis, as in peptone shock, rapid autolysis of the tissue protein may occur and thus lead to the same chemical changes in the animal body. Jobling and Peterson (6) have interpreted their finding as indicating "the cleavage of serum proteins (proteoses) through the peptone stage to amino-acids, and an intoxication by these peptones with a resulting cellular injury." I should not like to conclude from the results of my experiments whether it may be the peptone-like split products, as Jobling and others have maintained, which give rise to such a process and other symptoms in anaphylaxis, or whether it may be, as Weil (7) reported, the result of such hepatic lesions as are seen in poisoning by chloroform or phosphorus which involves the function and structure of that tissue. The problem must remain to be solved by further researches.

#### SUMMARY

1. Peptone intoxication is associated with a marked increase in urea nitrogen and also more or less in non-urea and amino nitrogen, thus confirming the results which have been reported by Whipple and Van Slyke.

2. The changes in the nitrogenous constituents of the blood in anaphylaxis are similar to those of peptone intoxication but more intense.

3. Anaphylaxis as well as peptone intoxication lead to an abnormally rapid autodigestion of tissue protein. The causative factors, as yet undetermined, are probably the same in both cases.

I wish to acknowledge my indebtedness to Prof. Dr. S. Mita for his suggestions and kind advice in carrying out this work, also to Prof. Dr. K. Katayama for his encouragement.

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## THE EFFECT OF QUININE ON THE NITROGEN CONTENT OF THE EGG ALBUMEN OF RING-DOVES

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v. Noorden (1) observed in mammals that prolonged dosage with quinine was accompanied by a marked diminution of the nitrogenous bodies which appeared in the urine. He concluded that under such prolonged dosage the nitrogen of the food and tissues is not dissipated so rapidly but is somehow conserved or stored in the body. In this laboratory Riddle and Anderson (2) investigated the following subject: When the body of a ring-dove produces eggs it separates from itself a large amount of protein for the formation of the egg-white and egg-yolk. Does the presence of quinine in the organism at the time of egg-formation affect the amount of protein which the body yields to the egg? From that investigation it was concluded that the *amount* or volume of albuminous substance expended by the body in the production of egg-white (and probably also of egg-yolk) is decreased under quinine. v. Noorden's conclusion was thus confirmed and one tissue or organ—the oviduct—was identified as a specific part of the body which shares in the conservation of nitrogen under quinine.

Riddle and Anderson studied only the relative amounts or quantities of albuminous material produced under dosage and made no determinations of the actual amount of nitrogen present in the normal and treated egg albumens.<sup>1</sup> They state (p. 99):

Whether there was an actual reduction of the nitrogen present is not definitely shown by our data since it is conceivable, even though not at all probable, that the reduction occurred only or chiefly in the amount of water present in the albumen.

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<sup>1</sup> Evidence was obtained (p. 100) indicating that in the egg-yolks the relative proportions of protein and lipoid were unchanged; the absolute amounts of both being diminished.



To learn definitely whether and how the nitrogen is affected in such egg albumens was the purpose of the present study. It may be stated at once that the result of this study shows that under quinine the amount of the egg albumen decreases; and that this diminished quantity certainly contains a smaller amount of nitrogen and a higher percentage of water than the normal albumen.

#### MATERIAL AND METHODS

Seven of the eleven birds used by Riddle and Anderson (2) were used for the present study. These birds were blond and white ring-doves and hybrids of these two forms. They were all a year older than at the time of the earlier experiment and for our purposes were, of course, being subjected to a second period of dosage. The advantages of using the same birds and at the same season of the year, and weighing and analyzing approximately the same number of clutches of eggs, are obvious. It should be noted, however, that previous dosage may have had some lasting effects on the birds and that it is perhaps too much to expect all of the birds precisely to duplicate the situation observed in the previous experiment.

The birds were all freely laying females, each penned with its own male mate. Pairs of eggs were usually obtained from each female at intervals of seven to ten days. The quinine was administered in the form of gelatin-coated quinine sulfate pills. The dosage,  $\frac{1}{4}$  grain at first and  $\frac{1}{2}$  grain later, was given twice daily—in the early morning and the late afternoon. During a period of four days following the laying of the first egg of the pair or clutch the dosage was omitted, as had been done in the earlier experiment, in order that the eggs might not be broken and the beginning of growth of the succeeding pair of yolks not too greatly delayed. Dosage was begun in March. The date of change of dosage (to  $\frac{1}{2}$  grain twice daily) varied slightly, but for most birds was about May 4. No blank dosing was considered necessary with these very tame birds, since earlier work had shown that the effect of handling is negligible.

The accurate weighing of the albumen, essential alike to accurate comparisons of the gross amounts of albumen produced and to a determination of percentage nitrogen based on per gram solids (see below) presents considerable difficulties and some possible inaccuracies.<sup>2</sup>

<sup>2</sup> During its passage down the oviduct the yolk is continually absorbing water from the albumen (Spohn and Riddle, (3) p. 405). After laying this loss continues and a further loss of water from the albumen to the air begins. The best that

Omitting any extended statement on these difficulties, however, we may note that the method adopted was to weigh the whole egg as soon as possible after laying,<sup>3</sup> steam it for eight minutes, cool in cold water for four, dry the shell and rapidly separate the yolk and egg-white with a clean blunt spatula. The yolk weight was next obtained; the albumen, kept meantime in a weighed and covered weighing bottle, was weighed on analytical balances. The albumen was dried in an oven at 105° and placed in a desiccator until used. From a comparison of the dry weight with the original weight the per cent of solids was determined. A sample for analysis consisted of approximately 0.5 gram of the combined *air dry* albumens from the two eggs of a clutch or pair. When no second egg of the clutch was laid a single egg was used alone.

The determination of the total nitrogen was made by the Kjeldahl-Gunning method and the distillate titrated against N/2 NaOH. From the total net weight of the albumen the actual weight in grams of the solids of the albumen was determined; and from this latter figure the weight in grams of nitrogen contained in each albumen was calculated. Although two weighed samples were usually combined to make one Kjeldahl sample, the percentage nitrogen determination thus obtained was applied *separately* to each of the two weighed samples, in order to have separate figures for the *absolute* weight of nitrogen of each individual egg. There is, of course, the possibility that the utilized portion of the combined samples did not consist of equal amounts of the two albumens, and that hence the figure for percentage nitrogen obtained was not equally typical of both samples. But if the admixture and manipulation is thorough and accurate this possibility is surely remote.

#### PRESENTATION OF DATA

The most important results can be found in condensed form in table 1. If the gross amounts of albumens of "before" and "during dosage" periods be compared it will be seen that this amount is diminished in

can be done is to make weighings on *all* eggs immediately after laying. When the preparation of the sample is delayed after weighing the following inaccuracies develop: The total weight of the egg is too small (0.005 to 0.003 gm. per hour); the total albumen is too small by the amount of the moisture lost to air and to yolk; the yolk is too large by the amount of moisture absorbed from the albumen.

<sup>3</sup> With four exceptions the eggs were examined within 5 minutes to 3 hours of laying. These four exceptional eggs were laid long after the usual time and hence were overlooked for a considerable number of hours. Three of these four eggs were from ♀ E97, whose exceptional record will be noted later.

five of the seven groups compared. In two groups (K459 and E97) it is increased.<sup>4</sup> In all of the seven groups the percent of water is increased during dosage. The percentage nitrogen per gram solids shows considerable fluctuations for these two periods. The amount of nitrogen in grams is decreased in five and increased in two (A347 and E97) of the "during" dosage periods.

Table 2 shows that the average amount of albumen in eggs (of five birds) from the "before" dosage period was 5.5924 gm.; for the "during dosage" period 5.4685 gm. The average percentage of solids was 10.81 in the earlier period and 9.83 in the "during dosage" period. The average amount of nitrogen was 0.0725 gm. per albumen for the "before" dosage period and 0.0661 for the "during dosage" period. These figures all exclude the record of two (E97 and 962) of the seven females.<sup>5</sup>

The amount and composition of the albumen produced after discontinuance of dosage, by birds which had been earlier subjected to prolonged quinine dosage, may next be considered. This "after dosage" period has been divided into three parts, as was done by Riddle and Anderson. Table 1 shows the result for the six individual birds producing such eggs; table 2 presents a summary for five of these. This summary indicates that the percentage nitrogen per gram solids is practically normal for the "after dosage" period as a whole. The percentage of solids, however, is markedly lower than before dosage. The actual amount of nitrogen per albumen is considerably decreased in the whole of the "after dosage" period although the total weight of albumen is only slightly decreased. In fact, in the "later" period the average weight for the individual albumen is slightly increased over the "before dosage" period (table 2).

The fact last mentioned above requires a further statement. In this "later" period the individual albumens are slightly heavier (solids are decreased) than in the control, or pre-dosing period, in spite of the fact that they were accompanied by smaller yolks (see table 1). The control yolks have an average (weighted) value of 1.900 grams, while those of the "later" period average only 1.841 grams. This confirms the view of Riddle and Anderson (p. 96) that there occurs "an excessive

<sup>4</sup> In both of these cases it will be seen that the whole egg weights of these control (or "before" dosing) eggs were abnormally small as compared with the control eggs produced by the other birds.

<sup>5</sup> The relative order of none of the above figures would be changed by including the record of these two females. The reasons for excluding the records of these females from table 2 will be given later.

TABLE 1  
Principal figures obtained from a study of the production of egg albumen, and of the percentage of nitrogen and water in the albumen, of seven ring-doves dosed with quinine sulfate

| NUMBER OF BIRD | DURATION OF DOSAGE | PERIODS AS RELATED TO DOSAGE | AVERAGE WEIGHT |       | AVERAGE           |                |                        | NUMBER OF N <sub>2</sub> DETERMINATIONS |         | AVERAGE                        |                         |
|----------------|--------------------|------------------------------|----------------|-------|-------------------|----------------|------------------------|---|---------|--------------------------------|-------------------------|
|                |                    |                              | Eggs           | Yolks | Albumens (weight) | Per cent water | Hours before weighing* |   |         | Per cent N <sub>2</sub> solids | N <sub>2</sub> in grams |
| 152            | March 9 to May 21  | Before                       | 14             | 1.805 | 5.7011            | 89.83          | 1.2                    | 7                                       | 11.33   | 0.0654                         |                         |
|                |                    | During                       | 18             | 7.956 | 5.4171            | 90.73          | 0.7                    | 9                                       | 12.29   | 0.0616                         |                         |
|                |                    | First after                  | 2              | 8.131 | 5.4999            | 90.80          | 0.5                    | 1                                       | 12.46   | 0.0619                         |                         |
|                |                    | Later                        | 4              | 8.349 | 5.6473            | 90.89          | 0.3                    | 2                                       | 12.44   | 0.0637                         |                         |
|                |                    | Last                         | 4              | 8.731 | 5.4304            | 90.93          | 1.4                    | 2                                       | 12.47   | 0.0608                         |                         |
| A347           | March 24 to May 19 | Before                       | 12             | 1.845 | 5.2167            | 89.47          | 0.8                    | 7                                       | 11.94   | 0.0639                         |                         |
|                |                    | During                       | 10             | 7.766 | 5.0344†           | 90.97†         | 1.1                    | 6                                       | 13.22   | 0.0593†                        |                         |
|                |                    | First after                  | 2              | 7.966 | 5.2186            | 91.16          | 0.5                    | 1                                       | 11.49   | 0.0527                         |                         |
|                |                    | Later                        | 4              | 8.094 | 5.1819            | 90.72          | 0.3                    | 2                                       | 12.08   | 0.0579                         |                         |
|                |                    | Last                         | 5              | 7.886 | 5.3655            | 90.34          | 1.1                    | 2                                       | 12.63   | 0.0641                         |                         |
| K459           | March 11 to May 16 | Before                       | 6              | 7.557 | 5.1862            | 89.95          | 1.0                    | 4                                       | 13.40   | 0.0701                         |                         |
|                |                    | During                       | 16             | 7.784 | 5.3267            | 90.32          | 1.1                    | 8                                       | 11.97   | 0.0620                         |                         |
|                |                    | First after                  | 2              | 7.528 | 5.2364            | 90.46          | 1.3                    | 1                                       | (16.99) | (0.0845)                       |                         |
|                |                    | Later                        | 4              | 8.191 | 5.6061            | 89.13          | 3.4                    | 2                                       | 10.99   | 0.0675                         |                         |
|                |                    | Last                         | 4              | 8.197 | 5.5012            | 90.39          | 0.6                    | 2                                       | 11.93   | 0.0633                         |                         |
| E106           | March 8 to May 18  | Before                       | 9              | 8.634 | 5.9673            | 88.06          | 1.2                    | 5                                       | 11.59   | 0.0791                         |                         |
|                |                    | During                       | 16             | 8.417 | 5.8560            | 89.44          | 1.0                    | 9                                       | 12.20   | 0.0748                         |                         |
|                |                    | First after                  | 2              | 8.612 | 5.9756            | 90.48          | 1.0                    | 1                                       | 12.42   | 0.0712                         |                         |
|                |                    | Later                        | 3              | 8.783 | 6.0326            | 90.56          | 0.4                    | 2                                       | 12.11   | 0.0693                         |                         |
|                |                    | Last                         | 5              | 8.527 | 5.7606            | 90.43          | 1.5                    | 3                                       | 12.07   | 0.0666                         |                         |



|     |                          |             |    |       |       |        |       |     |   |       |        |
|-----|--------------------------|-------------|----|-------|-------|--------|-------|-----|---|-------|--------|
| E97 | March 16<br>to<br>May 23 | Before      | 8  | 7.914 | 1.753 | 5.2361 | 89.51 | 3.8 | 4 | 11.67 | 0.0641 |
|     |                          | During      | 16 | 8.088 | 1.715 | 5.5054 | 89.37 | 1.6 | 9 | 11.86 | 0.0696 |
|     |                          | First after | 2  | 8.110 | 1.693 | 5.6030 | 90.15 | 0.8 | 1 | 12.10 | 0.0667 |
|     |                          | Later       | 4  | 7.496 | 1.561 | 5.0882 | 89.62 | 7.1 | 4 | 12.11 | 0.0636 |
| 903 | March 8<br>to<br>May 17  | Last        | 4  | 7.557 | 1.595 | 5.2251 | 90.37 | 1.1 | 2 | 11.42 | 0.0574 |
|     |                          | Before      | 12 | 8.953 | 2.269 | 5.7633 | 88.61 | 1.0 | 7 | 12.65 | 0.0858 |
|     |                          | During      | 12 | 8.536 | 2.056 | 5.5745 | 89.40 | 1.4 | 6 | 12.54 | 0.0724 |
|     |                          | First after | 2  | 8.815 | 2.041 | 5.4901 | 88.77 | 0.3 | 1 | 11.94 | 0.0721 |
| 902 | March 6<br>to<br>May 24  | Later       | 3  | 8.744 | 2.160 | 5.7107 | 90.15 | 0.4 | 2 | 12.13 | 0.0679 |
|     |                          | Before      | 16 | 9.153 | 1.877 | 6.1844 | 89.62 | 1.0 | 8 | 11.85 | 0.0768 |
|     |                          | During      | 14 | 8.396 | 1.645 | 5.8618 | 89.76 | 1.1 | 7 | 12.39 | 0.0703 |
|     |                          |             |    |       |       |        |       |     |   |       |        |

\* The average time interval between laying of egg and preparation of the albumen.

† For only 9 albumens.

or supernormal production of egg albumen . . . in this post-treatment period." These data (table 1) for "first after" periods also, in the main, support their conclusion. In the series of "last" eggs examined here, however, it seems that such influence has quite disappeared.

In any general consideration of the question of altered quantities of albumen per egg, in both the "during dosage" and "after dosage" periods, it must be borne in mind that the size of the egg-yolks—which is normally an effective part of the stimulus<sup>6</sup> to the secretion of the egg

TABLE 2

*Comparative summary of percentage nitrogen and amounts of solids and albumen (weighted averages for all birds exclusive of females 97 and 962)*

| AVERAGE                                      | BEFORE<br>DOSAGE | DURING<br>DOSAGE | AFTER DOSAGE |          |         |
|--|------------------|------------------|--------------|----------|---------|
|  |                  |                  | First        | Later    | Last    |
| Per cent N <sub>2</sub> per gram solids..... | 12.04            | 12.37            | (13.06)      | 11.93    | 12.28   |
| Per cent solids.....                         | 10.81            | 9.83             | 9.67         | 9.72     | 9.50    |
| Hours before weighing.....                   | 1.08             | 1.04             | 0.71         | 1.01     | 1.13    |
| Weight albumen in grams.....                 | 5.5924           | 5.4737           | 5.5067       | 5.6095   | 5.5198† |
| Weight N <sub>2</sub> in grams               | Expected*....    | 0.0725           | 0.0706       | 0.0713   | 0.0727  |
|  | Obtained†....    | 0.0725           | 0.0661       | (0.0684) | 0.0648  |

\* Amount of N<sub>2</sub> "expected" from the actually obtained amount of albumen; the figures given presume that the percent of solids, and percent N<sub>2</sub> per gram solids remain as in the period before dosage.

† These figures are weighted averages of amounts actually obtained. They do not check absolutely with calculations from the figures of rows one, two and four; this is because the high and low individual determinations of percentage N<sub>2</sub> and solids do not necessarily coincide with high and low absolute amounts of albumen.

‡ Applies to four birds. One of the five produced no eggs for this group.

albumen—is reduced in both of these periods. In the "during dosage" period the average (weighted) size of the yolks was only 1.788 grams (with egg size of 8.091 grams, and albumens of 5.4737 grams), while for the pre-dosing period these yolks weighed 1.900 grams (with egg size of 8.442 grams and albumens of 5.5924 grams. See third row of figures from bottom of table 2). The average difference in amount of albumen for these two periods is only 0.1187 gram; the average yolk size difference being 0.1200 gram. This would seem to indicate that the change in yolk size does account for the diminished albumen. It

<sup>6</sup> For discussion see (2, p. 99).

will be shown below that the "weighted averages" from which the above figures are derived fail to show the real situation. Unquestionably the size of the yolk is diminished as a result of the dosage with quinine as is shown for most of the birds of the present series and by all of those observed by Riddle and Anderson.

Since it can be questioned whether, on the basis of the figures given above, the diminished quantity of egg albumen secreted both during and after quinine is not wholly accounted for by the effect of the quinine in reducing yolk size the data of table 1 may be further consulted. For each bird studied it can there be clearly seen that the yolk size produced under quinine is associated with decidedly *smaller eggs* than are yolks of the same or similar size of the "before dosage" or "after dosage" periods. These decidedly smaller eggs must therefore signify smaller albumens for it is not possible to account for the difference as increase in shell under quinine. The data and conclusions of Riddle and Anderson on this matter are also in full accord with this conclusion. The same fact is further shown in the present data by a comparison of the "first after" and "during" periods. In all of these six possible comparisons the yolks were smaller in the "first after" period; nevertheless in four<sup>7</sup> of these "first after" periods the amount of albumen produced was greater than that of the "during dosage" period. Table 3 may be similarly consulted with like result. It thus becomes nearly certain that the amount of crude albumen or egg-white produced under quinine is less than the normal amount for the size of yolks then being produced and that this reduction is therefore directly due to the quinine.

We may next consider a further modification of the albumen which is certainly produced by the quinine. This concerns the smaller percentage of solids, and correspondingly larger amounts of water, present in albumen produced under dosage with quinine. The albumen of the "before dosage" eggs has 10.81 per cent solids; the "during dosage" 9.83 per cent (table 2). This means that the solids are reduced under the drug by 9.1 per cent of their normal value. This reduction continues moreover into the "after dosage" period for a number of weeks. v. Noorden (1) noted that the diminished excretion of nitrogen in the urine of mammals lasted a period of days after discontinuance of dosage.

We have already noted that the total nitrogen recovered from the "during dosage" period is clearly below that of the normal. We may

<sup>7</sup> If proper allowance be made for ratio of albumen to yolk (roughly 2:1) five of the six show the above situation.

now observe (last row, table 2) that this reduction of amount of nitrogen is continued through the "after dosage" period. Further, the differences between the "observed" and "expected" amounts of nitrogen are so large as to be clearly inexplicable as indirect results of smaller associated yolks. Here again an effect of the quinine on the nitrogen of the albumen seems unquestionable. Under quinine dosage the egg-albumen of the ring-dove receives more water and less solids than normally; and though these solids probably contain normal or slightly increased percentages of nitrogen, the absolute amounts of nitrogen in these albumens is reduced.

In table 3 is given the detailed record of data as obtained for one of the females. The record is essentially typical and it is not considered necessary to give similar individual records for the other females studied. From the data of this table the effects of the double-dosage of quinine may be observed in the six eggs laid on and after May 4. Calculation will show that during this double-dosage the average size of egg, yolk, albumen, percentage nitrogen per gram solids, and total nitrogen in grams, were all still further reduced beyond the point obtained from the lighter dosage. The figure for moisture, however, is here also slightly reduced. All other birds (including E97 and 962) with the exception of E106 gave essentially similar results for the effect of double-dosage. For the entire group of birds the percentage of water is further decreased in three and increased in four cases.

The condensed record obtained for female E97 has been given in table 1. The reasons for excluding her record from the general summary of table 2 may now be stated. The egg yolks of the "before dosing" period, and of the "later" and "last" periods (table 1) were found at the end of the present experiments to be so much below her previous record (see Riddle and Anderson, (2), table 2) as to cause much doubt as to the normality of this bird. Instead of the normal increase of yolk size which accompanies age (4, p. 391) these yolks had *decreased* in size by 12.8 per cent. Moreover the yolks of E97 failed most pronouncedly to increase in size in "after dosage" periods. Two other birds of this series showed slighter decreases in yolk size (962, decreased by 5.3 per cent; and E106, by 5.2 per cent) at the beginning and end of the present study as compared with their earlier records. These three birds were therefore all killed (September 1) about 50 days after the termination of the records here given in order to see whether *disease* was present and whether this could be responsible for the abnormal size of yolks. In view of the previously noted relations borne



TABLE 3  
*Details of record of female no. 152*

| PERIODS AS RELATED TO<br>DOSAGE | DATE OF<br>EGG | EGG<br>WEIGHT | YOLK<br>WEIGHT | WET<br>WEIGHT<br>AL-<br>BUMEN | HOURS<br>BEFORE<br>WEIGH-<br>ING | PER<br>CENT<br>N <sub>2</sub> PER<br>GRAM<br>SOLIDS | PER<br>CENT<br>WATER | N <sub>2</sub> IN<br>GRAMS |
|---------------------------------|----------------|---------------|----------------|-------------------------------|----------------------------------|---|----------------------|----------------------------|
| Before.....                     | 12 / 6         | 8.043         | 1.613          | 5.3417                        | 0.5                              | 8.65  | 90.30                | 0.0448                     |
|                                 | 12 / 8         | 8.823         | 1.925          | 5.5832                        | 2.0                              |   | 87.20                | 0.0618                     |
|                                 | 12/29          | 8.472         | 1.793          | 5.0380                        | 1.0                              | 12.71   | 94.20                | 0.0371                     |
|                                 | 12/31          | 8.648         | 1.775          | 5.5375                        | 1.5                              |   | 87.70                | 0.0865                     |
|                                 | 1/10           | 8.272         | 1.705          | 5.4336                        | 0.5                              | 10.03   | 90.54                | 0.0529                     |
|                                 | 1/12           | 9.150         | (2.025)        | 6.3886                        | 1.5                              |   | (84.80)              | 0.0973                     |
|                                 | 1/21           | 7.943         | 1.603          | 5.1951                        | 1.0                              | 10.18   | 90.15                | 0.0521                     |
|                                 | 1/23           | 9.237         | 2.088          | 6.2299                        | 1.5                              |   | 89.10                | 0.0691                     |
|                                 | 2/14           | 8.128         | 1.662          | 5.4884                        | 1.0                              | 12.02   | 91.15                | 0.0583                     |
|                                 | 2/16           | 8.457         | 1.688          | 5.7508                        | 1.3                              |   | 89.74                | 0.0709                     |
|                                 | 2/23           | 8.253         | 1.725          | 5.6818                        | 1.0                              | 12.78   | 90.25                | 0.0707                     |
|                                 | 2/25           | 9.168         | 2.060          | 6.0779                        | 1.5                              |   | 90.60                | 0.0730                     |
|                                 | 3 / 4          | 8.377         | 1.673          | 5.6671                        | 1.0                              | 12.95   | 90.87                | 0.0670                     |
|                                 | 3 / 6          | 9.123         | 1.940          | 6.4012                        | 1.0                              |   | 91.03                | 0.0743                     |
|                                 | Average        | 8.578         | 1.805          | 5.7011                        | 1.2                              | 11.33   | 89.83                | 0.0654                     |
| During.....                     | 3/14           | 7.370         | 1.690          | 4.8552                        | 0.5                              | 12.52   | 90.65                | 0.0568                     |
|                                 | 3/16           | 7.787         | 1.772          | 5.1732                        | 1.0                              |   | 91.61                | 0.0543                     |
|                                 | 3/23           | 8.023         | 1.598          | 5.6258                        | 0.5                              | 13.04   | 90.55                | 0.0762                     |
|                                 | 3/25           | 8.477         | 1.885          | 5.7945                        | 2.0                              |   | 92.99                | 0.0529                     |
|                                 | 4 / 2          | 7.672         | 1.465          | 5.4720                        | 2.0                              | 12.52   | 90.56                | 0.0646                     |
|                                 | 4 / 4          | 8.733         | 1.880          | 6.1000                        | 1.0                              |   | 90.40                | 0.0733                     |
|                                 | 4/10           | 7.382         | 1.523          | 5.0040                        | 1.0                              | 13.03   | 90.88                | 0.0594                     |
|                                 | 4/12           | 8.390         | 1.700          | 5.6841                        | 1.3                              |   | 91.71                | 0.0613                     |
|                                 | 4/18           | 7.945         | 1.605          | 5.5669                        | 0.0                              | 12.29   | 90.29                | 0.0664                     |
|                                 | 4/20           | 8.567         | 1.745          | 5.9530                        | 0.0                              |   | 90.98                | 0.0659                     |
|                                 | 4/26           | 7.700         | 1.550          | 5.3824                        | 0.0                              | 12.26   | 90.18                | 0.0647                     |
|                                 | 4/28           | 8.605         | 1.778          | 5.4753                        | 0.0                              |   | 90.52                | 0.0636                     |
|                                 | 5 / 4          | 7.748         | 1.620          | 5.2142                        | 0.5                              | 12.00   | 90.34                | 0.0604                     |
|                                 | 5 / 6          | 7.883         | 1.562          | 4.7318                        | 0.3                              |   | 90.93                | 0.0514                     |
|                                 | 5/13           | 7.623         | 1.600          | 5.0609                        | 0.5                              | 11.70   | 90.35                | 0.0571                     |
|                                 | 5/15           | 7.955         | 1.640          | 5.5880                        | 0.5                              |   | 87.42                | 0.0702                     |
|                                 | 5/21           | 7.478*        | 1.488          | 5.3010                        | 1.0                              | 11.30   | 90.60                | 0.0562                     |
|                                 | 5/23           | 7.870*        | 1.592          | 5.5271                        | 1.3                              |   | 91.24                | 0.0547                     |
|                                 | Average        | 7.956         | 1.649          | 5.4171                        | 0.7                              | 12.29   | 90.73                | 0.0616                     |
| First after.....                | 5/29           | 7.790         | 1.380          | 5.0711                        | 1.0                              | 12.46   | 90.90                | 0.0574                     |
|                                 | 5/31           | 8.478         | 1.700          | 5.9287                        | 0.0                              |   | 90.70                | 0.0664                     |
|                                 | Average        | 8.134         | 1.540          | 5.4999                        | 0.5                              | 12.46   | 90.80                | 0.0619                     |

TABLE 3—Continued

| PERIODS AS RELATED TO<br>DOSAGE | DATE OF<br>EGG | EGG<br>WEIGHT | YOLK<br>WEIGHT | WET<br>WEIGHT<br>AL-<br>BUMEN | HOURS<br>BEFORE<br>WEIGH-<br>ING | PER<br>CENT<br>N <sub>2</sub> PER<br>GRAM<br>SOLIDS | PER<br>CENT<br>WATER | N <sub>2</sub> IN<br>GRAMS |
|---------------------------------|----------------|---------------|----------------|-------------------------------|----------------------------------|---|----------------------|----------------------------|
| Later.....                      | 6/6            | 7.843         | 1.535          | 4.6925                        | 0.0                              | 11.97   | 90.39                | 0.0539                     |
|                                 | 6/8            | 8.443         | 1.700          | 5.9637                        | 0.0                              |   | 90.87                | 0.0651                     |
|                                 | 6/14           | 8.330         | 1.638          | 5.8578                        | 0.0                              | 12.91   | 90.88                | 0.0689                     |
|                                 | 6/16           | 8.780         | 1.842          | 6.0752                        | 1.0                              |   | 91.44                | 0.0671                     |
|                                 | Average        | 8.349         | 1.678          | 5.6473                        | 0.3                              | 12.44   | 90.89                | 0.0637                     |
| Last.....                       | 6/23           | 8.448         | 1.775          | 4.3691                        | 1.3                              | 12.52   | 89.84                | 0.0556                     |
|                                 | 6/25           | 9.153         | 1.997          | 5.7623                        | 1.3                              |   | 91.57                | 0.0608                     |
|                                 | 7/1            | 8.500         | 1.675          | 5.5453                        | 1.5                              | 12.42   | 91.08                | 0.0614                     |
|                                 | 7/3            | 8.823         | 1.872          | 6.0449                        | 1.5                              |   | 91.26                | 0.0656                     |
|                                 | Average        | 8.731         | 1.829          | 5.4304                        | 1.8                              | 12.47   | 90.93                | 0.0608                     |

\* Dosage of quinine doubled during this period.

by yolk size to the total amount of albumen this point is of considerable importance. The result of the post-mortem examinations of these three birds is given herewith:

♀ E97. Killed (September 1) for autopsy. Spleen much enlarged; with several firm tubercles throughout. Liver somewhat enlarged; texture soft. A very large cheesy tubercle in left lobe of liver at upper anterior border where it is also adherent to pericardium. Two tubercles on intestine, hard, apparently non-progressive. Oviduct medium functional. Largest ovum in left ovary about 3.5 mm.; plain detached traces (3 points) of right ovary. Some tuberculosis in left lung; well walled-off, apparently non-progressive. Pancreas and intestines extremely pale. Joints entirely free of tuberculosis. No lice present. Body not at all emaciated. Last egg laid August 12.

♀ 962. Killed for autopsy. This bird, obviously tubercular, had been removed from breeding pen on June 29. Body cavity entirely filled with purulent fluid. Very advanced tuberculosis in liver; this being enlarged, softened, and with numerous cheesy tubercles. Spleen enormously enlarged (about 3 grams). Oviduct small. Largest ovum in left ovary less than 1 mm. A trace of right ovary. Large tubercle in left lung; right lung healthy. Last egg laid May 23.

♀ E106. Healthy, killed for autopsy. Fully functional oviduct; two large ova in left ovary nearly ready to ovulate (9 and 11 mm.), also others nearly 5 mm. A trace of right ovary. Spleen and liver normal. A small single, cheesy, tubercular cyst or nodule at the tip of the right lung. Bird otherwise wholly normal in appearance. Bird had laid two eggs 5 and 3 days (August 28) before autopsy.

These records make it fairly evident that some at least of the abnormally small eggs of E97 were produced by a tubercular bird, and that

this tuberculosis was probably of the slowly progressive type that has been often observed in the birds of this collection. Moreover, as earlier noted, of the four instances in which the weights and preparations of individual samples were long delayed, three of these occurred among the eggs obtained from this bird. Such delays modify the results, and corrections for such delays are difficult and not wholly accurate. For all of the reasons already mentioned the record of E97 is excluded from table 2. The autopsy of female 962 shows a very advanced stage of progressive tuberculosis. Although the egg and yolk weights of this female were not far from normal for the "before dosage" period, her failure to produce any eggs in the "after dosage" period affords reason for excluding the data obtained from her, as has been done in table 2. The autopsy of E106—the general size of whose eggs was least abnormal throughout—shows on the other hand that this bird was practically or almost entirely unaffected and gives no warrant for the exclusion in table 2 of the data obtained from her.

Two further topics remain to be mentioned. There seems to be no report in the literature of any nitrogen determinations on doves' eggs other than those here reported. Pennington (5) has made nitrogen determinations on the egg albumen of two varieties of domestic fowl. The eggs for these determinations were collected under very nearly ideal conditions. With six to eleven determinations, each based on six to eighteen eggs, the nitrogen of the albumen (water-free) averages 14.28 per cent for Plymouth Rocks and 14.63 for Leghorns. The individual determinations ranged from 13.20 to 15.75 in the one case, and from 14.01 to 14.96 in the other. Our figures show averages varying around 12.00 per cent. The averages for the different periods among the several birds vary from 11.33 to 13.24, although in the individual records there are some wide exceptions. Some wide exceptions are also to be found among the individual determinations of amount of solids.

For the purposes of such a summary presentation as has been made, in table 2 particularly, our data would be more nearly ideal if equal numbers of eggs might have been obtained from each of the birds for each period. But from whatever standpoint the data are examined it seems clear that the nitrogen output of the albumen-secreting gland of ring-doves is diminished under quinine.

## CONCLUSIONS

Fresh-laid dove eggs contain about 12 per cent nitrogen per gram of solids.

The data of Riddle and Anderson on the reduction of egg size and yolk size under quinine treatment are further corroborated by the records of six of seven birds retested: Egg size and yolk size are decreased during dosage and increased after dosage is discontinued.

The normal quantity of (a more dilute) albumen is restored quickly after discontinuance of dosage.

Less albumen is produced during dosage than before. Relatively more (of a more dilute) albumen is produced after dosage is discontinued than during dosage.

The loss of weight or amount of albumen under quinine consists in *a*, a loss of total substance; and *b*, a disproportionate loss of solids.

The loss of solids is accompanied by a loss of nitrogen. When the amount of albumen is later increased, in the after-dosage period, the nitrogen does not increase in full proportion. The percentage of water remains high in albumen produced in these after-dosage periods.

It seems clear that dosage of ring-doves with quinine sulfate causes less than the normal amount of nitrogen to be released by the albumen-secreting gland of the oviduct during the secretion of egg-albumen.

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## THE NOSE-LICKING REFLEX AND ITS INHIBITION

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The chief and obvious purpose of the licking of the nose in animals is to clean its orifices. To accomplish that end the anterior part of the tongue must be freely movable and long, and the space which separates the nose from the mouth must be comparatively short. In animals in which these conditions are present, in dogs for instance, nose-licking is not merely an incidental phenomenon; it is a steady and apparently indispensable companion of the act of drinking, while the nasal orifices are mostly under the surface of the liquid. Furthermore, dogs get the liquid into their mouth not by the mechanism of suction, like in man, but by giving the anterior part of the tongue a spoonlike formation and throwing the liquid into the posterior oral cavity. Under these circumstances it certainly frequently occurs that some of the liquid is thrown into the nasal openings. These conditions obviously interfere with the respiration, and it is apparently a necessity for the animal, in order to facilitate its breathing, to frequently interrupt the drinking and cleanse the openings of the nose by means of licking.

The licking is accomplished by successive coördinated contractions of various voluntary muscles of the anterior part of the tongue which are under the control of the hypoglossus nerves. The entire procedure of the nose-licking makes the impression of being a conscious, voluntary act. Furthermore, the licking movements do not appear when the animal is under the influence of ether or chloroform anesthesia.

As far as we know, the physiological literature contains no indication that these movements may also be of a reflex nature. At the last meeting of the American Physiological Society we reported briefly on a mechanical method of anesthesia (this Journal, 1919, xlix, 120). The method consists in a carefully applied indirect concussion of the skull over the parietal bones. This procedure, when properly per-

formed, abolishes voluntary motions and all sensory reactions, without affecting the reflexes. For instance, strong pinching of the skin or strong electric stimulations of it or of exposed sensory nerve trunks (supra-orbital or lingual), leaves the animal perfectly quiet and without any evidence that it feels any pain. The reflexes, however, are very little affected, if any. The respiratory and vasomotor centers remain apparently intact; respiration continues in a normal rhythm, and the blood pressure remains unaffected; also the reflexes of these centers are unimpaired, for instance, stimulation of the central end of the vagus nerve causes inhibition of the respiration, and stimulation of the central end of the sciatic nerve causes an unmistakable rise of blood pressure; the eyelid and corneal reflexes appear to react in a normal fashion.

This signifies that in the indirect concussion of the brain, when properly performed, we possess a method which is capable of completely abolishing voluntary motions and sensations without perceptibly interfering with reflex actions. Employing this method, we tested the nature of the nose-licking act. The results which were obtained were constant; they are twofold and are very instructive.

We shall first mention the fact that *compressing in some way, for instance, by hemostatic forceps, the tip end of the nose or of the anterior part of the septum, causes a very characteristic nose-licking.* The appearance of this seemingly normal nose-licking movement in an animal which shows no other reaction to a pain-producing stimulus is a surprising sight. The nose-licking movements in such an animal are evidently of reflex origin, a fact which agrees with the previously mentioned observation that reflexes are not abolished under this method of mechanical anesthesia.

The second noteworthy observation is the fact that the nose-licking reflex occurs only after the cessation of the adequate stimulus. *During the pressure no attempt of nose-licking is made, may the pressure be ever so strong.* It seems to us that these observations express the facts that *during* the stimulation the nose-licking reflex is *inhibited* and that it may make its appearance only after the discontinuation of the stimulus. These phenomena were obtainable for many hours, that is, as long as the animal was under observation.

In other words, our experiments brought out the instructive facts *that the phenomenon of nose-licking is or may be a purely reflex act, and that this reflex mechanism consists of two parts: an inhibition of nose-licking during stimulation, and the setting in of the nose-licking movements soon after the discontinuation of the pressure stimulus.*

Obviously the afferent path of this reflex is located in the ophthalmic branch of the trifacial nerve, and the nerve fibers of the hypoglossus which convey the motor impulses to the anterior part of the tongue contain the efferent nerve fibers of this coördinated reflex. No doubt there is room for a further study of many details of this new reflex, and the mechanism may have to be considered later from various angles. There is, however, one point of view which we wish to discuss here.

It seems to us that our observations on the nose-licking reflex—namely, that it appears only after discontinuation of the compression of the tip end of the nose or the septum and that during this compression the reflex is inhibited—can be explained best by the following assumptions. First, the afferent path of this reflex consists of two antagonistic nerve fibers, nerves, the stimulation of which causes an impulse for the excitation of the muscles performing nose-licking; and nerve fibers, the stimulation of which causes an inhibition of the motor part of this reflex. Second, that when both kinds of the reflex fibers are stimulated simultaneously, the response of the reflex inhibitory fibers predominates to such a degree as to completely obscure the presence of the impulse of the reflex excitation. Third, that the response of the reflex inhibitory fibers to the stimulation possesses either a very weak after-effect, or none at all, while the stimulation of the reflex exciting fibers continues its effect after cessation of the stimulation, to such an efficient degree as to bring out the nose-licking reflex in a definite fashion. In other words, we interpret our phenomenon by assuming that when both (antagonistic) nerve fibers are stimulated simultaneously by compression, the effect on the inhibitory fibers predominates during stimulation; but the nose-licking reflex makes its appearance after the discontinuation of the stimulation by virtue of the efficient after-effect of the reflex excitation impulses.

The foregoing several assumptions are sufficiently supported by facts well known in the physiology of the nervous system. For instance, the assumption that a simultaneous stimulation of antagonistic nerves may bring out the effect of the inhibitory impulses during stimulation while the exciting action may outlast the stimulation and appear after its cessation, in consequence of the after-effect of the exciting impulse, is well illustrated by the relations of the vagus and accelerans nerves in their action upon the heart. As is well known, stimulation of the peripheral end of the vagus causes inhibition, while stimulation of the accelerans nerve causes an acceleration and augmentation of the heart



beats. Now when both nerves are stimulated simultaneously, and the stimulus is also discontinued simultaneously, the heart stops beating during stimulation, while after discontinuing the stimulus, the heart beats more frequently and strongly than before the stimulation. This is due to the experimentally well-established facts that the inhibitory response of the vagus nerves predominates during stimulation and that after cessation of the stimulus a long after-effect of the accelerating nerves comes to the fore. In other words, we have here a well-established instance of results of simultaneous stimulation of two antagonistic nerves in which the inhibitory impulse responds during stimulation and the response of the exciting nerves appears after cessation of the stimulus due to an after-effect of the latter type of nerves. However, it must be borne in mind that in this instance the mentioned characteristic results take place in the periphery, within the heart, and not, as is the case in the nose-licking reflex, within a center located in the central nervous system.

On the other hand, the assumption that a single mechanical stimulus may cause simultaneously inhibitory and exciting reflexes in the spinal cord is illustrated by the facts discovered by Sherrington, namely, that a mechanical stimulus of a flexor muscle may cause simultaneously an excitation of the flexor and an inhibition of the extensor muscles (and *vice versa*)—Sherrington's "reciprocal innervation." But here again this instance differs from our observation on the nose-licking reflex, in that the stimulation of both antagonistic nerve fibers causes effects only during stimulation and not after its cessation, and that the effects of the stimulation become manifest in different groups of muscles; while in the nose-licking reflex, the effect of simultaneous stimulation of the antagonistic nerves becomes manifest in one and the same muscle group, and further, at different times, the exciting reflex effect appearing after the cessation of the stimulus, while the inhibitory reflex effect is active during stimulation.

Another illustrating fact is to be found in the observations of Kroecker and Meltzer on the propagation of the peristaltic wave within the esophagus. Throughout the entire length of the gullet the inhibitory action runs ahead of the action which causes the successive contractions of that organ. But it must be pointed out that in the deglutition mechanism the mentioned phenomenon becomes manifest in muscle fibers which are not under the control of volition; we have no definite knowledge as to the rôle which inhibition may play in the buccopharyngeal part of the mechanism of deglutition.



We may perhaps also refer here to the mechanism of the "self-regulation" of respiration. According to Herring and Breuer, distention of the lung causes an inhibition of the inspiratory and an excitation of the expiratory muscles, while collapse of the lung causes an inhibition of the expiratory and an excitation of the inspiratory muscles. In this theory it is assumed that the collapse of the lung is a stimulus and a specific one for the expiration: in other words, distention and collapse are different stimuli; each stimulus acting separately and specifically on the two antagonistic parts of the respiratory mechanism. Meltzer suggested (Du Bois-Reymond's *Arch. f. Physiol.*, 1892, 340) that in the mechanism of "self-regulation" there is only one stimulus for both antagonistic parts of the respiratory function; it is the distention of the lung which stimulates simultaneously both sets of antagonistic nerves; but both sets of nerve fibers differ in their response to the same stimulus by two characteristics, namely, the responses of the inspiratory fibers *a*, predominate during the stimulation (distention) and *b*, possess very little or no after-effect; while the impulses in the nerve fibers which control the expiratory mechanism are obscured during the stimulation but possess a long and efficient after-effect which becomes manifest after the discontinuation of the stimulus (distention). In other words, the inspiration is due to the predominating response to the stimulation of the inspiratory nerves during the distention, and the expiration is due to the after-effect of the impulses carried by the expiratory nerves which are dormant during the distention. According to this theory of respiration, the elements of the reflex mechanism for the function of respiration are to a great degree similar to those which we have assumed as underlying the nose-licking reflex. In the mechanisms of both reflexes the mechanical stimulus affects two antagonistic sets of nerve fibers simultaneously, but the effect of stimulation of one set of fibers predominates during stimulation and the stimulation carries no after-effect; while the impulses of the antagonistic nerves are dormant during stimulation, but become manifest after discontinuation of the stimulation on account of the long and efficient after-effect.

We believe that the element of the after-effect of stimulation is an important factor in the mechanisms of many functions. However, experimental physiology has hitherto completely neglected the study of the rôle which the after-effects of stimulations may actually play in the functions of the animal body.

## SUMMARY

Indirect concussion of the brain may abolish voluntary movements and the sensation of pain, without affecting reflexes. Indirect concussion, when properly carried out, may offer a useful method for bringing out the presence of new reflexes, especially those of the brain.

Nose-licking, at least in dogs, is a reflex act, the motor part of which, however, may, as in many other reflexes, also be performed voluntarily.

In an animal under mechanical anesthesia, induced successfully by indirect concussion of the brain, a nose-licking reflex may readily be brought about by compression of the tip end of the nose or the anterior part of the septum. This reflex is characteristic in that it appears only *after* the discontinuation of compression; it does not appear *during* the compression.

This characteristic behavior is explained by the assumption that the compression stimulates simultaneously two antagonistic sets of nerve fibers, reflex exciting and reflex inhibitory nerves. The influence of the impulse of the reflex inhibitory fibers predominates during the stimulation (compression); the impulses carried by the reflex exciting fibers are dormant during the stimulation but become manifest after discontinuation of the stimulus by virtue of the long and efficient after-effect of this set of nerve fibers.

## INFRA-RED RADIANT ENERGY AND THE EYE

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A number of years ago speculation was rife regarding the possibility that the absorption of infra-red radiant energy by the eye-media resulted in eye-fatigue. In fact, statements were made by eminent men that irritation and fatigue in the eye were due largely to this thermic effect. These statements were widely quoted with the result that many have accepted this explanation of the source of irritation and fatigue of the eyes under artificial illumination. Although the assumption may be proved eventually to be true, the author is unaware of any definite experimental foundation at the present time. Without declaring for or against this assumption, the author, several years ago, computed the amounts of energy absorbed in the eye-media under various conditions of illumination and also the energy-densities throughout the optical path in the eye. As a consequence two papers (1), (2) were published where they would reach those interested in lighting.

More recently there has developed quite extensively an opinion that eye-glasses, especially in the industries, should not transmit infra-red radiation. Therefore, it appears worth while to present some of these data within convenient reach of the physiologist and ophthalmologist who doubtless are best fitted to supply experimental evidence regarding the possibility of infra-red radiation causing visual discomfort, eye-fatigue or permanent injury to the eye-media. At present infra-red radiation stands convicted purely upon circumstantial evidence, and although it is not the intention to present a brief for or against this radiation, it appears desirable to show the type of data upon which the conviction is based.

Crookes (3) inferred "that it is to the heat rays rather than to the ultraviolet rays that glass-workers' cataract is to be ascribed" because these rays (ultra-red) are "present in the radiation from molten glass in far greater abundance than the ultraviolet rays." A number of questions of fundamental importance immediately arise. First, and of

primary interest, is the question of the existence of glass-blowers' cataract. The author has not encountered any qualified individuals in the glass industry who admit that cataract is prevalent among glass-blowers. Furthermore, even though such prevalence be admitted for the sake of analysis, there is still the possibility that the causes when traced to their source may lead the investigator far from the molten glass into other conditions of working or living (4). Infra-red radiation may be found to be effective in causing cataract but possibly only in conjunction with some other agency or condition. In fact, it may be completely exonerated in the case of glass-workers because ultraviolet radiation is emitted by molten glass as Crookes himself determined. Spectrograms made by him on photographic plates extended into the ultraviolet as far as  $0.3345 \mu$  when the exposure was prolonged sufficiently, but because of the low intensity of ultraviolet radiation compared with that of infra-red radiation, Crookes inferred that the blame must be attached to the latter. This is unphilosophical even when viewing the situation superficially and is extremely so when considering the chemical activity of ultraviolet radiation with the comparative ineffectiveness of infra-red radiation in this respect.

The properties of radiant energy are not well defined as to wave-length. For example, the chemical effect of ultraviolet radiation varies with the wave-length and this variation varies with the chemical process. Furthermore, it is misleading, for example, to state that ultraviolet radiation is chemically active; that visible radiation arouses the sensation of luminosity; and that infra-red radiation is "heat" energy. Either directly or indirectly these various radiations have many properties in common. When measured as heat energy, radiations of all wave-lengths are alike,—that is, they are readily converted into heat energy by absorption. A statement that infra-red radiation is the cause of cataract or eye-fatigue carries with it the possibility of condemnation of visible radiation on the same score because of certain similarities of the two radiations.

As shown later, if infra-red radiation is detrimental owing to its heating effect under conditions which do not "burn" the skin or eye-membranes, then sunlight, owing to its enormous intensity, must be looked upon with suspicion. Even the visible radiation in sunlight is quantitatively large owing to the extreme intensity, and if the thermic effect of radiation is harmful, then sunlight is dangerous even when the water-vapor of the atmosphere has absorbed all the infra-red radiation.



Perhaps one of the reasons for the growing belief that infra-red radiation is harmful to the eyes may be found in attributing to this energy a destructive ability similar or analogous to that of ultraviolet energy. But such a property is not established for infra-red. In fact, it appears to be even less destructive to animal tissue than some of the visible radiation.

Notwithstanding this line of reasoning and the lack of direct experimental evidence, infra-red radiation has been condemned so often that many now believe it to be injurious. In fact, this conviction is so seriously accepted in many quarters that it is gaining rapidly and bids fair to be very generally accepted by those interested in eye-protection. This is illustrated for example by a recent technical paper (5) containing excellent data under the title, "Glasses for protecting the eyes from injurious radiations." Although the authors in their introductory remarks call attention to the lack of proof "that infra-red rays have other than a thermic (if any) effect," the data presented largely pertain to infra-red radiation. To the indiscriminating the title of the paper containing, for the most part, data pertaining to the infra-red radiation, carries with it a strong implication if not a complete conviction that infra-red radiation is harmful to the eyes even for intensities too low to "burn" (in the ordinary sense) the skin and eye-media.

When considering vision, the infra-red radiant energy is at least useless and it is well to dispose of it if this can be done without too much expense and without injustice commercially. The brief discussion presented in the foregoing paragraphs does not aim to exonerate infra-red radiation but to focus attention upon the lack of experimental evidence. Many are now capitalizing this (at present) unfounded suspicion and therefore this is an important problem confronting the physiologist.

The data presented in this paper do not directly reach the root of the problem but they are of considerable importance. Energy quantities and densities in the eye-media are established herein and should aid the physiologist who is interested in the question. This paper is confined purely to the physical aspects of spectral energy-distribution in illuminants, of the absorption by the eye-media, of optical laws, of luminous efficiency of illuminants, etc. The radiation from the theoretical "black-body" at various temperatures is considered and industrial processes involving high temperatures may be compared in terms of these data because most bodies emitting radiant energy by virtue of their temperature may be placed at least approximately on the black-body scale. In fact, it is enlightening and not difficult to arrange

industrial processes approximately on a temperature scale according to the approximation of their emitted radiation to that of the theoretical black-body.

Any one familiar with the spectral distribution of energy in the radiation from ordinary light-sources is certain that less energy per lumen is incident upon the eye as the temperature of the radiator is increased, provided that the light is due to purely temperature radiation. The computations in this paper will be confined to purely temperature radiation or radiation from bodies which deviate but little from it. Just what relative amounts of energy are absorbed in the various eye-media and how the amount of absorbed energy varies with the temperature of the source have not been determined, although Vogt (6) has described elaborate experiments which show roughly the transmission of the eye-media in various regions of the spectrum. His results roughly confirm the more refined experiments of Aschkinass (7). Fortunately, Aschkinass has clearly established the transmission of the eye-media throughout a wide spectral range and has obtained other data which enable certain computations to be made. He found that the various eye-media transmitted the visible and infra-red rays in the same manner as like thicknesses of water. The only discrepancies found were for the transmission of the cornea for the visible and "near" infra-red rays. The differences found in that case he attributed to the film which rapidly forms over the dead cornea, rendering it less transparent, for the absorption-bands were well defined and in the same position as for water.

The intensity of radiation after traversing any depth,  $d$ , can be computed from the following equation:

$$I' = Ie^{-xd}$$

where  $I$  and  $I'$  are the original and final intensities respectively,  $e$  is the base of Napierian logarithms, and  $x$  is the extinction-coefficient. This can be further simplified for purposes of calculation thus

$$\frac{I'}{I} = T = e^{-xd}$$

or

$$\text{Log } T = -xd \log e$$

where  $T$  is the transmission-coefficient. If  $I$  is taken as unity, of course the value of  $I'$  is numerically equal to the transmission factor. Aschkinass gives a table of extinction-coefficients for water for radiant energy of

wave-lengths from  $0.45\mu$  to  $8.49\mu$ . It is thus possible to compute the transmission of the various eye-media for this range of wave-lengths. Aschkinass did this for the total eye but not for the various media. For the purpose of computation, thicknesses of water corresponding to the various eye-media were taken. These are reproduced in table 1.

TABLE 1

*Thicknesses of water corresponding to thicknesses of various eye-media*

|                         | <i>Cm. of water</i> |
|-------------------------|---------------------|
| Cornea.....             | 0.06                |
| Aqueous humor.....      | 0.34                |
| Crystalline lens.....   | 0.42                |
| Vitreous humor.....     | 1.46                |
| Total depth of eye..... | 2.28                |

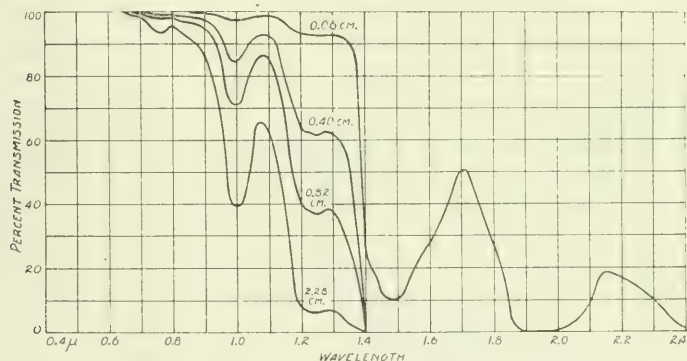


Fig. 1. Transmission of various layers of water corresponding to the eye-media.

Spectral transmission-curves were initially computed for four thicknesses of water, namely, 0.06 cm., 0.4 cm., 0.82 cm. and 2.28 cm. The first thickness corresponded to the cornea, the next to the cornea plus the aqueous humor, and so on. Obviously, the amount of energy absorbed in the aqueous humor, for example, is readily found by obtaining the absorption of the first two thicknesses and taking their difference. This was the general procedure. The spectral transmission curves of the four thicknesses of water are shown in figure 1.

The next point of interest was to apply these transmission-curves to the curves representing the spectral energy-distribution of black-bodies at various temperatures and also to those of various illuminants. This was done by multiplying the ordinates of the spectral energy-curves by

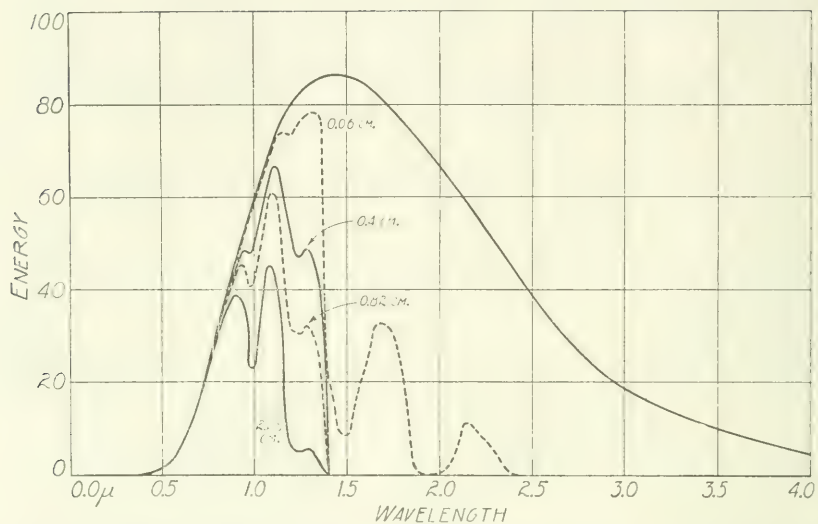


Fig. 2. Spectral transmission of radiant energy from a 4-watt-per-candle carbon lamp through various layers of water.

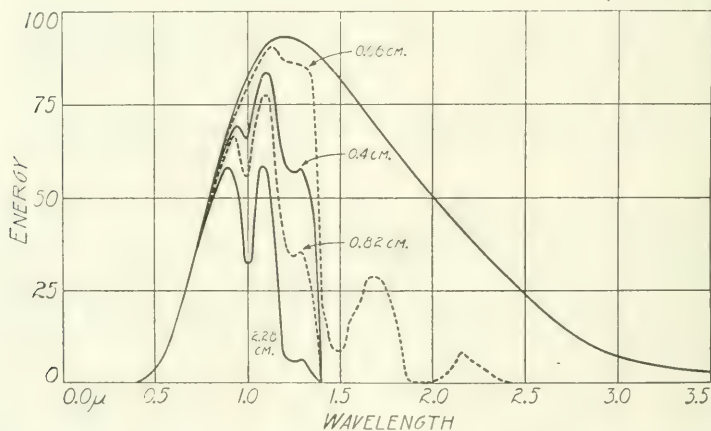


Fig. 3. Spectral transmission of radiant energy from a tungsten lamp (7.9 lumens per watt) through various layers of water.



the corresponding transmission-factors. Figures 2 and 3 show the results of this process with the spectral energy-curves of a 4-watt-per-candle carbon incandescent lamp and that of a 1.25-watt-per-candle tungsten lamp (7.9 lumens per watt) respectively. The numbers on the curves represent the thickness of water. For example, the percentage of total energy radiated from the carbon lamp which is transmitted by the cornea is found by obtaining the ratio of the area under this spectral transmission-curve (0.06 cm.) to the total area under the energy-distribution curve. The difference between this and unity gives the absorption of the cornea. The same process with the curve, 0.4 cm., gives the percentage of energy transmitted by the cornea and aqueous humor.

TABLE 2  
*Percentage of radiant energy absorbed in the eye-media*

| SOURCE                          | PERCENTAGE OF TOTAL ENERGY ABSORBED IN |         |          |          |        |               |      |                |
|---------------------------------|--|---------|----------|----------|--------|---------------|------|----------------|
|                                 | Water of depth                         |         |          |          | Cornea | Aqueous Humor | Lens | Vitreous Humor |
|                                 | 0.06 cm.                               | 0.4 cm. | 0.82 cm. | 2.28 cm. |        |               |      |                |
| Black body at 2000° absolute..  | 68.8                                   | 80.6    | 83.8     | 89.7     | 68.8   | 11.8          | 3.2  | 5.9            |
| Black body at 2500° absolute..  | 51.7                                   | 63.3    | 68.3     | 76.7     | 51.7   | 11.6          | 5.0  | 8.4            |
| Black body at 3000° absolute..  | 38.5                                   | 49.8    | 55.7     | 65.1     | 38.5   | 11.3          | 5.9  | 9.4            |
| Black body at 4000° absolute..  | 22.8                                   | 31.7    | 37.2     | 45.9     | 22.8   | 8.9           | 5.5  | 8.7            |
| Black body at 5000° absolute..  | 13.0                                   | 19.6    | 23.4     | 30.4     | 13.0   | 6.7           | 3.8  | 7.0            |
| 4-w. p. c. treated carbon lamp. | 64.1                                   | 77.3    | 81.0     | 87.9     | 64.1   | 13.2          | 3.7  | 6.9            |
| 1.25-w. p. c. tungsten lamp.... | 50.4                                   | 64.5    | 70.5     | 80.0     | 50.4   | 14.1          | 6.0  | 9.5            |

Obviously, the difference between the two transmission-factors gives the percentage of total energy absorbed in the aqueous humor, and so on. These percentages are found in table 2 and plotted in figures 4 and 6 for black-bodies at temperatures ranging from approximately 2000 to 5000 degrees absolute. At temperatures above 5000 degrees, the ultraviolet energy becomes appreciable, so no computations are given for higher temperatures. The curves would rise again for temperatures beyond 5000 degrees absolute. The energy of shorter wave-length than  $0.35 \mu$  radiated from a black-body at 5000 degrees absolute was found to be 3.8 per cent of the total energy.

Though the eye-media are found to transmit the visible and infra-red rays in the same manner as water, this is not true for ultraviolet radiation. Water is transparent to short-wave radiation far into the extreme ultraviolet. In fact, no noticeable absorption was found for

any of the ultraviolet radiation from the quartz mercury arc. It has been concluded by some that near ultraviolet radiation is chiefly absorbed in the eye-lens owing to the fact that it strongly fluoresces under the influence of these rays. This conclusion has been strongly confirmed by spectro-photographic evidence. However, for black-body temperatures below  $5000^{\circ}$  absolute the ultraviolet rays need not be considered from the standpoint of the temperature effects due to absorption of this energy. In figure 4 are plotted the percentages of total black-body radiant energy absorbed by the various eye-media. It will be noted

TABLE 3  
*Absorption of radiant energy in watts per lumen in the eye-media*

| SOURCE                                     | WATTS PER LUMEN ABSORBED IN |         |          |          |        |               |        |                |
|--|-----------------------------|---------|----------|----------|--------|---------------|--------|----------------|
|  | Water of depth              |         |          |          | Cornea | Aqueous Humor | Lens   | Vitreous Humor |
|  | 0.06 cm.                    | 0.4 cm. | 0.82 cm. | 2.28 cm. |        |               |        |                |
| Black body at $2000^{\circ}$ absolute..... | 0.153                       | 0.179   | 0.186    | 0.20     | 0.153  | 0.026         | 0.007  | 0.013          |
| Black body at $2500^{\circ}$ absolute..... | 0.04                        | 0.049   | 0.053    | 0.061    | 0.04   | 0.009         | 0.004  | 0.008          |
| Black body at $3000^{\circ}$ absolute..... | 0.012                       | 0.0156  | 0.0174   | 0.0202   | 0.012  | 0.0036        | 0.0018 | 0.0028         |
| Black body at $4000^{\circ}$ absolute..... | 0.0035                      | 0.0049  | 0.0057   | 0.0071   | 0.0035 | 0.0014        | 0.0008 | 0.0014         |
| Black body at $5000^{\circ}$ absolute..... | 0.0015                      | 0.0023  | 0.0027   | 0.0035   | 0.0015 | 0.0008        | 0.0004 | 0.0008         |
| 4-w. p. c. treated carbon lamp.....        | 0.247                       | 0.297   | 0.312    | 0.338    | 0.247  | 0.05          | 0.0015 | 0.026          |
| 1.25-w. p. c. tungsten lamp.....           | 0.064                       | 0.082   | 0.089    | 0.101    | 0.064  | 0.018         | 0.007  | 0.012          |

that for the cornea these percentages rapidly decrease with increase of temperature of the source, but much less rapidly for the aqueous humor, while the percentages of absorbed energy are a maximum for the lens and vitreous humor at about  $3500^{\circ}$  absolute. (See table 2). This would hardly be foreseen without computation. It is seen that most of the energy is absorbed in the outer portion of the eye. In reality, this absorption rapidly decreases as the depth of the absorbing layer increases. This, of course, is to be expected from the exponential character of the foregoing equation relating thicknesses of the eye-media with the transmission-factors.

So far only percentages of energy absorbed have been considered. However, it is important to reduce the data to a common basis, that is,

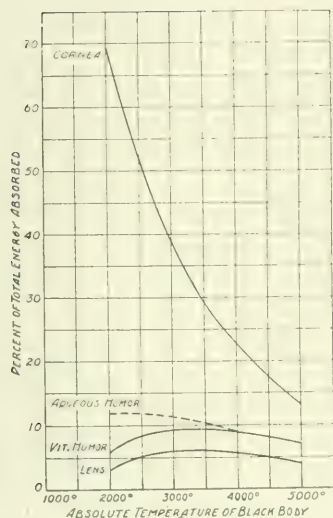


Fig. 4

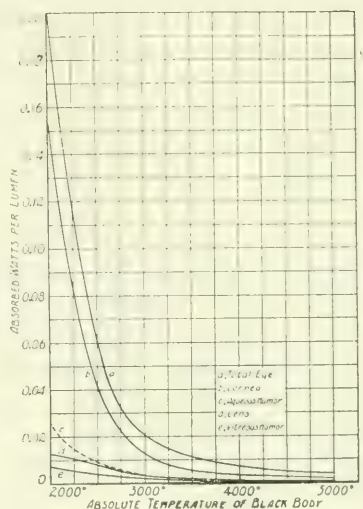


Fig. 5

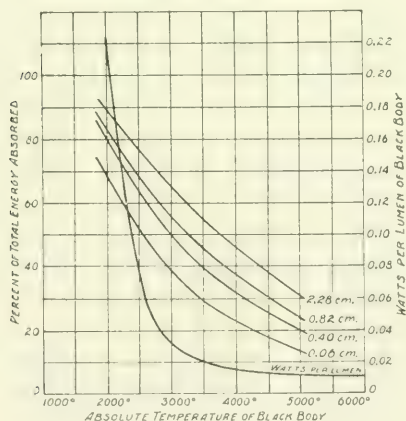


Fig. 6

Figs. 4, 5 and 6. Percentage of total radiant energy absorbed in various eye-media, watts absorbed in eye-media per lumen in usual percentage of light, and percentage of total radiated energy absorbed in various layers of water.

to find the actual watts absorbed per lumen. This was readily done by combining the foregoing percentages with the lumens per watt of the various sources. In figure 6 are plotted the values of watts per lumen for the black-bodies at various temperatures. Multiplying these values by the corresponding values from the curves in figure 4, the actual watts absorbed per lumen are found in each case. These values are presented in table 3 and are plotted in figure 5. Curve *a* represents the absorption by the total eye (2.28 cm. of water); curve *b*, that by the cornea. Curves *c*, *d* and *e* represent, respectively, the absorption by the aqueous humor, lens and vitreous humor. These curves indicate the actual power absorbed in the eye-media per lumen of light flux in the entering beam. The computations take into consideration only a beam of light of such dimensions that it enters the pupil; however, the exterior portions of the eye must absorb much energy in rays which could not possibly enter the pupillary aperture. The cornea and aqueous humor, besides absorbing most of the energy in the useful beam, no doubt absorb a great deal more energy.

It is thus seen that the outer layer of the cornea absorbs a large portion of the energy which is not active in producing the sensation of light, and, as is to be expected, the absorbed energy per lumen of light flux incident upon the retina rapidly decreases with increase of temperature of the source. There would be an increase again for temperatures higher than about  $5000^{\circ}$  absolute due to the increasing amount of short-wave radiation. This, however, is not of interest here, because a large amount of the short-wave radiation would not be tolerated on account of its destructive effect. It will be noted that about thirty times as much energy is absorbed in the total eye per lumen of tungsten light as per lumen of light from a black-body at  $5000^{\circ}$  absolute. This same ratio would hold approximately for sunlight if it were not for the moisture in the atmosphere which absorbs much of the infra-red rays before they reach the earth and therefore the eye. This is perhaps fortunate considering the enormously greater intensities of illumination encountered in daylight. For instance, according to F. E. Fowle (8), the amount of precipitable water existing in the form of water-vapor between the top of Mount Wilson and the outer limits of our atmosphere during fair weather from June to November, 1910 and 1911, was found to vary from 0.2 cm. to 2.8 cm. The average quantity present was 0.69 cm.

Another point worthy of consideration is the energy-density at various points in the eye along the optical path. Obviously, owing to



the optical system through which the radiant energy passes, the energy-density varies in different parts of the eye. Where the energy is more concentrated the more serious effects might be expected.

Utilizing the foregoing and some additional data, the transmission of various depths of the eye (measured from the anterior surface of the cornea) is readily obtained. The spectral transmission-curves for radiation from a black-body at various temperatures and from a carbon and a tungsten lamp are shown in figure 7. Neglecting atmospheric absorption, which will be discussed later, the radiation from the sun can be assumed, for comparison purposes, to be approximately similar to that of a black-body at  $5000^{\circ}$  absolute. In order to obtain an idea of the energy-density in various parts of a beam of light passing through the eye, it is necessary to determine the path of the beam. Obviously, the path of the useful beam is slightly different as the eye is accommodated for near or distant vision. Data regarding the refractive indices, thicknesses and curvatures of the various surfaces of the eye-media as

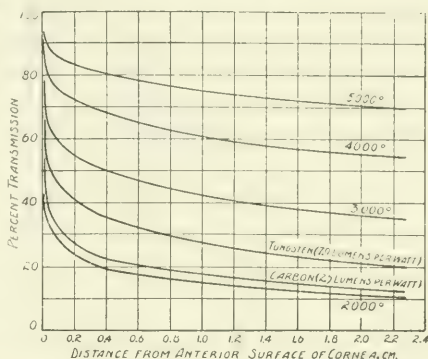


Fig. 7. Transmission of various depths of the eye for the radiation from different sources.

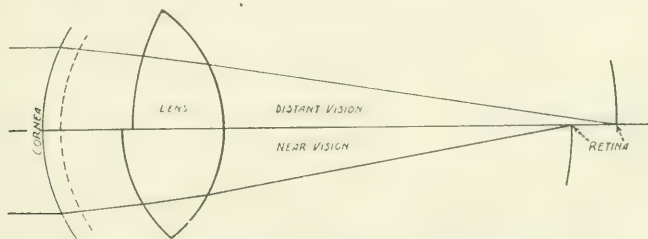


Fig. 8. Path of light in the eye (small object).

determined by Helmholtz were used. Only the beam that enters the pupillary aperture (in this case assumed to be 5.8 mm. in diameter) was considered. Computations give the paths for near and distant vision for a small object as shown in figure 8. The cross-section is considered circular as determined by the pupillary aperture. An image 0.01 mm. in diameter upon the retina would normally be formed

by a circular disk 10 mm. in diameter viewed at a distance of about 15.5 meters. The refraction from the cornea into the aqueous humor is disregarded owing to the very small difference between their refractive indices; that is, for the purpose of computation, the aqueous humor is considered as extending to the anterior surface of the cornea. The thickness of the cornea is shown to scale by the dashed line drawn parallel to its anterior surface. The eye becomes shorter and the lens thicker when accommodated for near vision.

The mean energy-density in various vertical layers of the eye-media under the above conditions is shown in figure 9. Curve *A* represents

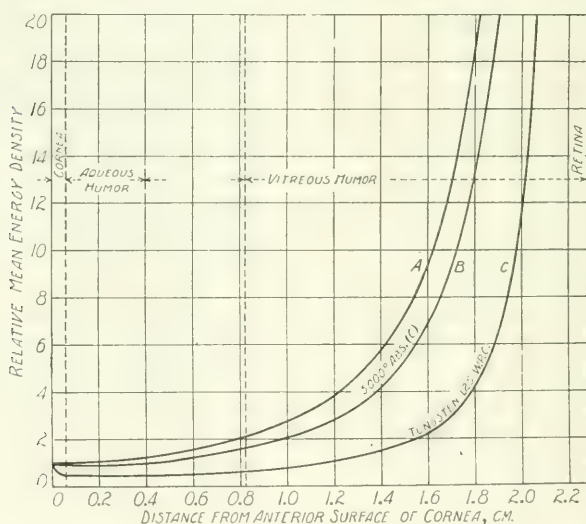


Fig. 9. Energy-density in the useful beams of light from a small source (distant vision).

the relative energy-density in various parts of the beam if there were no absorption of energy by the eye-media. If the small disc be diffusely and totally reflecting and be illuminated by radiation from a black-body at 5000° absolute, the mean energy-density in various parts of the path of the beam is represented by curve *B*, after allowing for absorption. Curve *C* represents the conditions when the illuminant is a vacuum tungsten lamp operating at 7.9 lumens per watt. It is seen, as might be expected, that the mean energy-density becomes relatively high near the retina. There is quite a uniform mean energy-density, however, for more than half of the distance of the path in the eye. This is

due to the fact that the absorption of energy in the eye-media approximately overcomes the concentrating effect of refraction. In the case of curve *C*, this absorption more than overcomes any concentrating action for one-half the distance. In order to compare curves *B* and *C* on a basis of mean energy-density per lumen, the ordinates of curve *C* must be multiplied by a factor approximately equal to thirty. In other words, the energy-density is about the same for a beam of tungsten radiation (7.9 lumens per watt) containing one unit of light flux as for a beam of radiation (of the same area of cross-section) from a black-

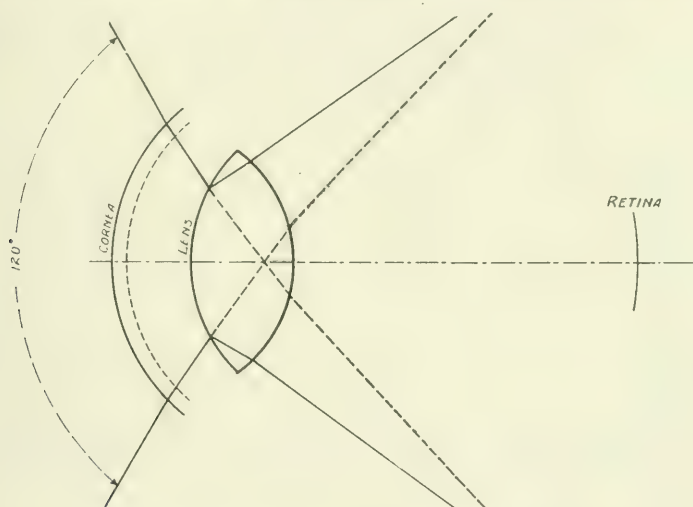


Fig. 10. Path of light in the eye (extended object).

body at 5000° absolute containing about thirty units of light flux. The energy-density curves are practically the same for near vision as for distant vision, and hence they have not been plotted.

Before discussing the foregoing further, it is of interest to consider the other extreme case—namely, when the object subtends a large angle. The entire visual field subtends approximately a solid angle of 120°. The useful beam of radiation included within a solid angle of 120° at the eye is shown by the full lines in figure 10, when the eye is accommodated for a reasonably near vision. If the object that is being viewed be illuminated with the same density of radiation of the same spectral character as the object considered in figure 8, it is obvious that the brightness of the retinal image will be the same and a much greater

amount of energy will pass through the pupillary aperture. In other words, the energy-density at the retina will be the same in the two cases, but the energy-density in the lens and anterior section of the eye will be many times greater in the case of the extended source. The ratio of the energy-densities in the plane occupied by the pupillary aperture will be approximately equal to the ratio of the solid angles subtended by the sources. In the two cases considered, when the brightnesses of the retinal images are equal, the energy-density in the pupillary plane for the case of the extended source is several million times greater than in the case of the small source. This is shown diagrammatically in figure 11 for equal energy-densities at the retina—that is, for equal brightnesses of the retinal images. Curve *D* represents the condition for the extended source and curve *E* for the small source.

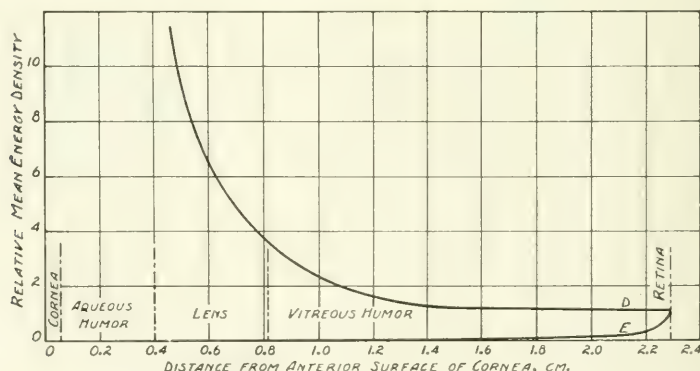


Fig. 11. Energy-density in the useful beams of light from sources subtending large, *D*, and small, *E*, solid angles.

The foregoing conditions are of importance in many cases of vision if eye-fatigue or cataract is to be attributed to the absorption of energy. For instance, a small area of molten glass or metal may appear quite harmless. A larger area of equal brightness should appear equally harmless, but, as is evident from the foregoing, the actual energy-density in various parts of the beam would be far different in the two cases. In the case of a large area the energy-density in the lens would be many times greater than in the case of the small area. In ordinary vision there are many cases of extended areas of comparatively high brightness, such as a snow field, a desert, the sky, the walls of a room and pavements.



It is of interest to consider the possible effect of sunlight (radiation approximately like that presented for a black-body at  $5000^{\circ}$  absolute), but in doing so allowance must be made for the absorption of radiant energy by the water-vapor in the atmosphere. It has been observed that water-vapor is somewhat more transparent to the sun's radiation than is water in the liquid state. Assuming that the absorption of the water-vapor existing in the atmosphere is equivalent to a layer of the liquid 1.5 cm. in thickness, and with due consideration of the relative luminous efficiencies of a black-body at the apparent temperature of the sun and of the tungsten lamp, it is found that approximately a hundred times as much energy is absorbed by the eye per lumen of tungsten light as per lumen of sunlight. But it is not uncommon to find sunlight intensities far greater than a hundred times that encountered with artificial light under working conditions. In fact, the ratio of actual working intensities is often greater than 1000 to 1. This indicates that eye-fatigue and cataract should be quite noticeable under natural lighting conditions if they can be attributed to an energy effect. In fact, cataract is quite prevalent in India, but in this case, according to the work of Burge (4), the cause might be traced to an accumulation in the liquids of the body of something which so modifies the lens protein that energy of certain short wave-lengths can precipitate it, thereby causing opacity.

To summarize, it is shown that when viewing luminous objects of small area (subtending a small solid angle) there is no serious concentration of energy in the eye-media until the retina is approached. However, when viewing extended objects (large solid angle) there is a relatively much greater energy-density in the lens and anterior parts of the eye than in the posterior portions. When the retinal images are of the same brightness, there will be a very much greater energy-density in the lens when viewing an object subtending a large solid angle than when the object subtends a small angle if the spectral character of the illuminant and the intensity of the illumination are the same. This indicates that large sources of radiation of a relatively low visual brightness might be effective in forming cataract or causing eye-fatigue if the "absorption-of-energy theory" is correct. In fact, if the deterioration of the lens is due to ultraviolet rays, the latter might be present in such small amounts as to appear harmless, but when it is recalled that the energy-density in the lens is very high when viewing extended objects, such as the sky, pavements, surfaces of molten glass, metal, etc., it appears to be possible that the ultraviolet rays still might be present in

sufficient amount to do damage. From this standpoint sunlight, owing to the greater intensities encountered, appears to be probably as effective in producing cataract and eye-fatigue as ordinary artificial illuminants and some industrial processes, even after allowing for the higher luminous efficiency of the former and the absorption of energy by the water-vapor present in the atmosphere.

It is not claimed that the data in this paper settle the question of the influence of energy absorption in the eye-media. The object has been to show in what relative quantities the energy is absorbed in the various parts of the eye and also the energy-density in the path of the useful beam of radiation in order that those interested in eye-fatigue and cataract might have these quantitative data available.

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## STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

### V. THE ISOLATED HEART AS AN INDICATOR OF ADRENAL SECRETION INDUCED BY PAIN, ASPHYXIA AND EXCITEMENT

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Knowledge of the conditions under which the endocrine glands become active or manifest increased activity is important for several reasons. Such knowledge is valuable as an extension of our acquaintance with a realm of physiology which is still largely unexplored. It permits correlated studies of other bodily processes which vary under the same conditions. And it gives a basis for inquiry into the functions performed by the endocrine glands, for the service of an organ should reasonably be looked for in relation to the times of its special activity. With such ideas in mind, the present series of studies was entered upon.

In two papers published in 1911, Cannon, in collaboration with de la Paz (1) and with Hoskins (2), brought forward evidence that the adrenal medulla was stimulated to secrete by emotional excitement, by "pain" and by asphyxia. Adrenal secretion had previously been proved to be subject to sympathetic stimulation by way of the splanchnics; and as excitement, pain and asphyxia were conditions well recognized as accompanied by sympathetic activity (manifested, e.g., by inhibition of digestive functions), an attendant adrenal secretion was naturally to be expected. In a series of papers which followed these first two, experiments were described showing that adrenal secretion was serviceable in lessening muscular fatigue (3) and in accelerating coagulation of the blood (4). An interpretative paper (5) pointed out that excitement, pain and asphyxia were conditions which in natural existence would commonly be associated with struggle, and that the visceral changes, including adrenal secretion, which accompany these three states, would be useful in great muscular effort. This interpretation presented a new view of the function of the sympa-

thetic division of the autonomic system and of the adrenal medulla in important bodily adjustments.

Within the past few years both the evidence on which the foregoing interpretation was based and the interpretation itself have been seriously questioned. In an extensive series of papers, Stewart and Rogoff have reported apparently careful quantitative studies on the rate of adrenal discharge, and have drawn the conclusions that the discharge is continuous, that in any animal it is approximately constant, and that the supposed variation is dependent on the rate at which the blood flows through the lumbo-adrenal veins (6). They found no increase of secretion in pain (7), asphyxia (8) or emotional excitement (9). More recently Gley and Quinquaud have also examined experimentally adrenal secretion and have come to the decision that adrenin is not secreted in sufficient amount to be carried effectively to the organs on which it may act, and that therefore no true physiological adrenalinemia exists (10). The sharp difference between the views put forth by Cannon and his coworkers and the ideas supported by these later investigators warrants a reëxamination and a thorough testing of the evidence for adrenal secretion in pain, asphyxia and excitement.

#### REVIEW OF THE POSITIVE EVIDENCE

1. *That adrenal secretion is induced by sensory stimulation.* In the original tests Cannon and Hoskins (2) made use of rhythmically contracting segments of rabbit intestine suspended lengthwise in a glass cylinder through which oxygen was passed. The segment, when not surrounded by the blood to be tested, was bathed in Ringer's solution. The test blood, the cylinder and the fresh Ringer's solution were all kept at body temperature in a common bath. The blood to be tested was taken before and after the experimental procedures by passing a catheter through a nick in the femoral vein into the iliac and thence into the inferior vena cava anterior to the entrance of the lumbo-adrenal veins. A thread tied tightly around the catheter marked the point to which it was inserted and permitted reinsertion to the same point in subsequent sampling of the blood. The position of the catheter opening, which was at one side, was kept constant by attention to the position of the knot in the thread. Thus both the control blood and the blood after stimulation were taken as exactly as possible from the same region. Under these circumstances normal blood removed before stimulation of the central end of the sciatic nerve caused no inhibition



of the rhythmically contracting intestinal segment, whereas that removed afterwards produced a marked relaxation. The conclusion was drawn that the adrenal glands are affected through nervous channels when a sensory trunk is strongly excited and that they then pour into the blood stream their secretion.

The foregoing conclusion was supported a year later (1912) by Anrep who found that the denervated limb or kidney at first expands but later quickly contracts when the central end of the cut sciatic nerve is stimulated (11). If the adrenal glands were removed or the splanchnic nerves were cut, the phase of contraction disappeared. Since the organs (limb or kidney) were denervated, the only factor which could cause their contraction in the presence of a rise of general blood pressure must be some agency brought by the blood stream; and since the phenomenon disappeared on exclusion of the adrenals, the conclusion was drawn that adrenal secretion, poured out in consequence of reflex stimulation through the splanchnics, produced the observed vasoconstriction.<sup>1</sup> The observations of Anrep on the denervated limb have recently been confirmed by Pearlman and Vincent (12).

The following year (1913) Levy reported the incidental observation that after both stellate ganglia had been removed and both vagus nerves cut, stimulation of the sciatic nerve occasioned irregularity of the heart (13). He also noted that excitation of the peripheral end of the cut splanchnic would cause the same cardiac changes, and that they did not occur if the adrenal gland was removed on the stimulated side. He therefore concluded that on sciatic stimulation the denervated heart was being affected by adrenin discharged reflexly.

In 1917 Florovsky (14) undertook an investigation of a strange fact previously observed by Ostrogorsky, which was that if the cervical sympathetic and the chorda tympani nerves are severed, and the secretory effect of a dose of pilocarpin is disappearing, sciatic stimulation causes a considerable increase in the flow of saliva. The effect was so striking that he looked for a third nerve to the submaxillary gland but could not find it. Under the conditions described by Ostrogorsky, Florovsky succeeded in producing augmented salivary secretion

<sup>1</sup> In the same year with Anrep's observation, Elliott reported (*Journ. Physiol.*, 1912, xliv, 406) exhaustion of the adrenal gland with intact nerve supply when the sciatic nerve was stimulated periodically for four hours. Since Elliott's methods were concerned with a quantitative assay of the amount of adrenin left in the gland after stimulation, and not directly with adrenal secretion, his results will not be considered in the present review of the evidence.

by stimulating the peripheral end of the cut splanchnic and by intravenous injection of adrenin. He also confirmed Ostrogorsky's observation of an augmented flow after sciatic stimulation. This reflex secretion did not occur, however, if both adrenal glands were extirpated, or if one was extirpated and the vein of the other obstructed, or if both splanchnic nerves were cut. He concluded, therefore, that the anomalous secretion from the denervated submaxillary gland is due to adrenal secretion resulting from reflex stimulation.

The foregoing evidence, involving tests made on blood removed from the body, and tests made in the body on the denervated limb, the denervated kidney, the denervated salivary gland and the denervated heart, are harmonious in testifying to a reflex discharge of the adrenal glands when a sensory nerve is stimulated.

2. *That adrenal secretion is induced by asphyxia.* In their examination of the effect of asphyxia on adrenal secretion, Cannon and Hoskins (2), in 1911, used the same methods that were employed for testing the effect of sensory stimulation. In the course of the examination it was discovered that *extreme* asphyxia would cause a change in the blood which would produce the same effect as adrenin on the beating intestinal strip, i.e., inhibition, and this even though the adrenal glands were carefully removed or the circulation confined to the region above the diaphragm. This observation indicated the necessity for careful control at the time the asphyxial blood was taken. Accordingly, after *moderate* asphyxia, there was removed from the femoral vein blood which should serve as a control sample of the systemic venous flow below the entrance of the lumbo-adrenal veins; and at as nearly as possible the same time another sample was removed from the inferior vena cava at a point anterior to the opening of these veins. This latter blood caused the typical inhibition indicating the presence of adrenal secretion, whereas the control femoral blood, like the vena cava blood taken before asphyxia, failed to cause inhibition. Through the use of the control, therefore, the presence of an accessory factor, simulating the action of adrenin, was ruled out. Consequently the conclusion was drawn that asphyxia results in secretion of the adrenal glands.<sup>2</sup>

<sup>2</sup> At about the same time that the paper by Cannon and Hoskins was published, Starkenstein (Zeitschr. Exper. Path. Therap., 1911, x, 78) reported that in one rabbit asphyxia caused nearly an abolition of the color reaction of the adrenal gland connected with the central nervous system, whereas the other gland, with its nerve severed, showed a good color reaction. In 1912, Borberg (Scand. Arch.

In 1912 Anrep (11) noted that a decrease in volume of the denervated limb and denervated kidney occurred during asphyxia, in spite of a general rise of arterial pressure, just as he had seen it occurring as a consequence of sciatic stimulation. This vascular constriction appeared, however, only when the adrenal glands were connected with the circulation and the splanchnic nerves were intact. When the adrenal glands were out of circulation, asphyxia caused some rise of arterial pressure, though less than in the intact animal, but no constriction in the vessels of the denervated limb or kidney. He concluded, therefore, that the adrenal glands are excited during asphyxia. These observations of Anrep on the constriction of the vessels in the denervated limb were at once confirmed by Itami (15), who found that it did not occur after transection of the cord. Since the constriction was not due to the direct action of  $\text{CO}_2$  on the vessel wall, nor to reaction of the vessels to an increased internal pressure, he interpreted the result as due to increased adrenal secretion.

In 1914 Gasser and Meek, while making observations on a dog with stellate ganglia removed and the vagi cut, noted, when the animal was asphyxiated for 30 seconds, an acceleration of the heart beat amounting to 92 beats per minute (16). Now, under ether anesthesia, the blood vessels of the adrenal glands were tied. After recovery from the operation, asphyxia lasting 90 seconds caused an acceleration of only 8 beats per minute.

In 1917 Gley and Quinquaud found an amount of adrenin in adrenal venous blood obtained during asphyxia considerably in excess of that obtained when the animal was undisturbed (17). Using the rise of blood pressure as a test, they determined that from 4 to 8 cc. of the asphyxial adrenal blood were equivalent to 16 cc. of the blood before asphyxiation. In their experiments injection of 20 cc. of blood from the inferior vena cava, taken above the adrenal veins after 3 or 4 minutes of asphyxia, caused a rise of arterial pressure from 24 to 45 mm.

Physiol., xxviii, 124) quoted Fridericia as having performed six experiments on guinea pigs poisoned with an excess of  $\text{CO}_2$  with some diminution of the chrome reaction in the glands. In the same year, Kahn (Arch. f. d. gesamt. Physiol., 1912, cxlvi, 578) reported asphyxia in monkeys as causing a marked difference in the adrenin content of the two adrenal glands, one of which was removed before asphyxia, the other afterwards. The adrenin present was quantitated by the use of Laewen's preparation. Since these observations, although they support the view that asphyxia causes adrenal secretion, are really assays of the amount of adrenin left in the gland after asphyxia, and do not give direct indication of adrenal activity, they will not be further considered in the present paper.



higher than that produced by injecting an equal quantity of cava blood taken from the same level before asphyxia.<sup>3</sup>

The foregoing evidence which, like that obtained after sensory stimulation, was the result of studies by various observers using a variety of methods, is harmonious in leading to the conclusion that adrenal secretion is increased by the asphyxial state.

3. *That adrenal secretion is induced by excitement.* In the experiments on the influence of emotional excitement, performed by Cannon and de la Paz in 1911 (1), the methods employed were similar to those used by Cannon and Hoskins. The only differences were that the animals did not receive a general anesthetic and that the catheter was introduced under local anesthesia. Controls were obtained in every instance. As the original records show, after emotional excitement the blood drawn from the inferior vena cava anterior to the opening of the adrenal veins repeatedly caused inhibition of the beating intestinal strip, whereas that removed before excitement had no such effect. Since excitement after removal of the adrenal glands did not yield this result, and since the effective blood lost its inhibitory power when exposed to oxygen (a procedure known to destroy adrenin), the inference was drawn that adrenal secretion is stimulated by great emotion.

These conclusions were confirmed in 1913 by Hitchings, Sloan and Austin (18), who used the same method to obtain blood and the same test for adrenin that Cannon and de la Paz had used. They found that after great fear and rage had been induced in a cat by the attempt of a muzzled dog to fight it, the adrenin reaction was clearly demonstrable. The reaction did not occur, however, if the splanchnic nerves had been previously severed.

In 1918 Redfield reported that in the horned toad nervous excitement causes a contraction of the melanophores in the denervated skin, a reaction which does not occur after the removal of the adrenal glands (19).

In addition to these direct observations on the stimulating effect of strong emotion on adrenal secretion, there were other observations having inferential value. In 1914 Cannon and Mendenhall, after show-

<sup>3</sup> Gley and Quinquaud express the opinion that the experiments of Cannon and Hoskins were indecisive in determining the effect of asphyxia on adrenal secretion, because relaxation of the intestinal strip could be induced by blood removed from the asphyxiated animal after the adrenals had been excised. They seem not to have paid attention to the control observations which Cannon and Hoskins were careful to make (see p. 402).



ing that clotting of the blood is hastened by stimulation of the splanchnic nerves, found that great excitement will cause the same effect (4). The evidence which they brought that injected adrenalin shortens the clotting time, that when the splanchnic nerves are stimulated the adrenal glands are necessary for the effect, and that excitement induces faster clotting only so long as the splanchnic nerves are intact, was confirmatory of the view that excitement causes adrenal discharge.

In 1915 Lamson noted that injection of adrenalin would cause a polycythemia, and that emotional excitement, such as fear and rage, would likewise cause it (20). If an animal was frightened after removal of the adrenal glands, however, there was no increase in the red count. Lamson observed that asphyxia had the same effect as fright and that removal of the adrenals prevented the customary increase seen after asphyxiation.

By both direct and indirect testimony, offered by different observers using different methods, the evidence is concordant that emotional excitement is accompanied by increased secretion of the adrenal medulla.

All of the observations cited above, leading to the conclusion that adrenal secretion is increased in pain, asphyxia and excitement, were on record before the negative results of recent investigations were published. These positive data have all been consistent, they were obtained by a number of quite independent workers, and they were the outcome of a variety of operative procedures each differing from those yielding the negative results. In view of these facts it would appear that this body of cumulative testimony deserved more consideration than it received, and warranted a comparison of experimental methods.

#### A CONSIDERATION OF CRITICISMS OF THE CATHETER METHOD

In criticism of the catheter method used by Cannon and Hoskins, Stewart and Rogoff declare first, that the results obtained by it are valid only if the blood flow is assumed to be constant during the whole experimental period; and second, that the method does not permit any judgment on this point (7). Thus, if there be a continuous secretion of adrenin undisturbed by reflex stimulation, as they maintain is the case, there could be an increased concentration only if the blood flow through the adrenal vessels were retarded. There is another possibility, however, which should be considered. The blood flow through the adrenal vessels might be *increased*. Strong sciatic stimu-

lation has a well-known pressor effect. This may be due largely to reflex splanchnic stimulation. But there is no evidence that splanchnic stimulation causes constriction of adrenal vessels. Indeed, the careful observations of Burton-Opitz and Edwards (21) have shown that stimulation of the splanchnic nerves causes a greater blood flow through the adrenal vein, a result which Biedl had previously noted (22). With a heightened general blood pressure and at least no constriction of the adrenal vessels, the blood flow through these vessels must necessarily be increased. Under these circumstances, on the basis of Stewart and Rogoff's argument, the adrenin would be more *dilute* rather than more concentrated in the adrenal blood. A faster flow in the inferior cava which might accompany the higher arterial pressure would still further dilute the secreted adrenin. With heightened arterial pressure, therefore, the conditions which would prevail in the inferior cava anterior to the adrenal veins would be highly unfavorable for demonstrating a greater concentration of adrenin, if adrenal secretion were constant and unvarying. The positive evidence which was obtained that adrenin is actually *concentrated* in the circulating blood at this point in times of stress indicates, definitely, an increased secretion from the glands.

The suggestion that the positive results obtained by Cannon and his collaborators might have been due to a fortunate location of the eye of the catheter (7) seems to have been made with disregard for the care exercised in making control observations under precisely the same conditions before and after stimulation.

Stewart and Rogoff's few attempts to obtain reactions from intestinal strips by use of blood taken by catheter from the inferior cava were unsuccessful (7). They explain their lack of success by supposing that the adrenal secretion was too highly diluted by cava blood. Without direct observation of their technique it is difficult to suggest the reason for their failure to obtain the positive results undoubtedly demonstrated by the catheter method. It may be stated, however, that the method is difficult and exacting, and that not until after some experience with it did it begin to yield us positive results.

#### THE DENERVATED HEART AS AN INDICATOR OF ADRENAL SECRETION

The difficulties encountered in testing for adrenin in blood removed from the body render desirable a simpler method which will yield reliable results in the hands of any competent experimenter. In 1917 Cannon (23), making use of the hint offered in the incidental observations of

Levy (13) and of Gasser and Meek (16), suggested the employment of the completely denervated heart to demonstrate an increase of adrenin in the circulating blood.<sup>4</sup>

This is a preparation which is likely to be highly serviceable in the further elucidation of adrenal function. In the first place, the preparation has important advantages dependent on testing the blood while it is still in the body—advantages which were praised by Stewart and Rogoff (7), but which were lacking in their use of the intestine and uterus as indicators. These advantages are: security against loss of adrenin in manipulation, avoidance of a development of the pressor property of clotted blood, exclusion of the possible effects on secretion of loss of blood or adrenin from the organism, and finally the possibility of direct quantitative comparison of adrenal secretion induced by successive stimulations. Further, the denervated heart is an organ highly sensitive to adrenin; intravenous injection of adrenalin at the rate of 0.001 mgm. per k. per minute has increased the heart rate as much as 28 beats per minute. Moreover, the method permits a graphic record from which may be judged the latent period and the duration of secretion of the adrenal glands in consequence of stimulation. And finally, the necessary operation (severance of the vagus nerves and removal of the stellate ganglia between the first two ribs on either side) is so simple that anyone inclined to doubt that more adrenin is secreted, in consequence of reflex or other stimulation, may readily make the test.

*Sensory stimulation.* In a cat under urethane, with vagi cut and stellate ganglia excised, stimulation of the central end of the cut sciatic will cause the heart rate to increase in some instances as much as 50 beats a minute.<sup>5</sup> Comparisons of the increased rate due to sciatic stimulation with the effects of adrenalin (quantitated as base) injected intravenously indicate that the range of reflex adrenal secretion lies

<sup>4</sup> At the same time Cannon reported (*Science*, May, 1917, xlv, 463) that the denervated heart revealed an increase of adrenal secretion after sciatic stimulation or asphyxiation, and promised a full report of the experiments in this *Journal*. Absence from the United States for nearly two years has unavoidably delayed the complete paper until this time.

<sup>5</sup> Many years before Levy's observation, Hunt noted (this *Journal*, 1899, ii, 444) that stimulation of a sensory nerve would cause cardiac acceleration after all cardiac nerves were divided, and that the same result followed stimulating the peripheral splanchnic. It differed from the acceleration following sympathetic stimulation by beginning slowly, i.e., about ten seconds after the start of stimulation. It is interesting to observe that this period is almost exactly that required for the distribution of adrenal secretion by the circulating blood.



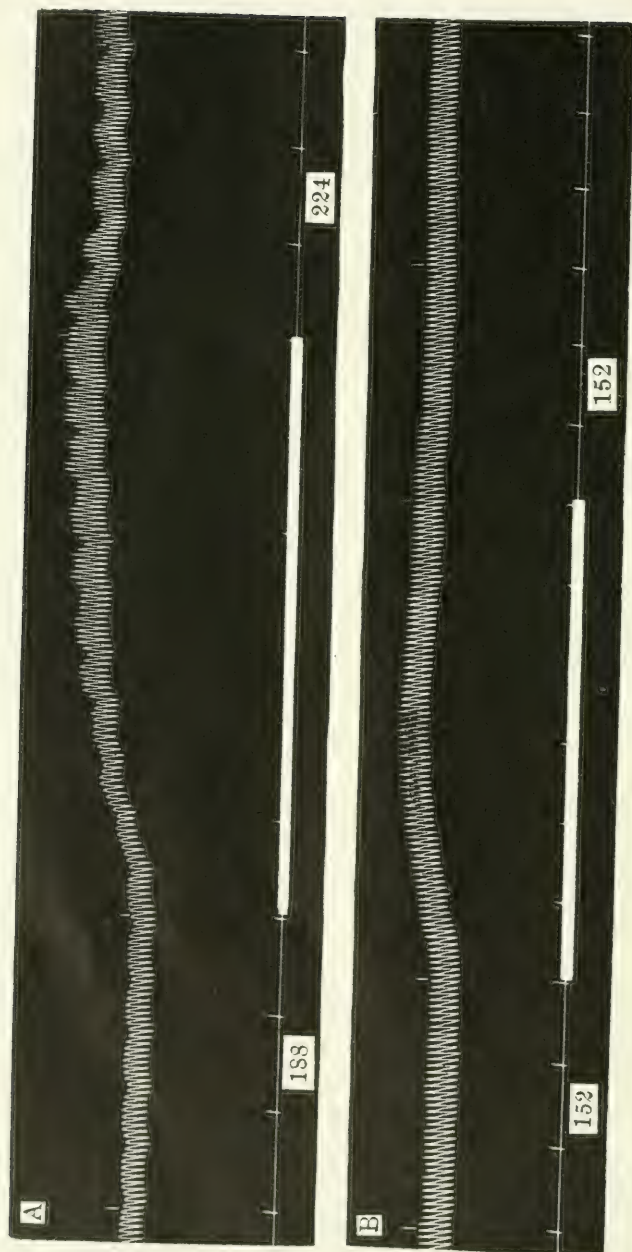


Fig. 1. Records of the beat of the denervated heart, membrane manometer. Time intervals, 5 seconds.  
A, Sciatic stimulation 30 seconds, 2:21. Increase of rate from 188 to 224 per minute. (Adrenal glands tied off, 2:52).  
B, Sciatic stimulation 30 seconds, 3:10. No increase of rate.



TABLE 1

*Examples of increased rate of the denervated heart on sciatic stimulation*

| DATE          | TIME  | SCIATIC STIMULATION                              | RATE BEFORE | RATE AFTER | INCREASE PER MINUTE |
|---------------|-------|--|-------------|------------|---------------------|
| March 21..... | 2.58  | 30 seconds                                       | 220         | 264        | 44                  |
|               | 3.04  | 30 seconds                                       | 220         | 256        | 36                  |
|               | 3.15  | 15 seconds                                       | 212         | 240        | 28                  |
|               | 3.16  | Splanchnics cut                                  |             |            |                     |
|               | 3.25  | 30 seconds                                       | 184         | 192        | 8                   |
|               | 3.28  | 15 seconds                                       | 184         | 192        | 8                   |
| March 23..... | 2.16  | 30 seconds                                       | 176         | 228        | 52                  |
|               | 2.21  | 30 seconds                                       | 188         | 224        | 44                  |
|               | 2.52  | Adrenal glands tied                              |             |            |                     |
|               | 3.07  | 15 seconds                                       | 152         | 156        | 4                   |
|               | 3.10  | 30 seconds                                       | 152         | 152        | 0                   |
| April 6.....  | 2.31  | 30 seconds                                       | 216         | 240        | 24                  |
|               | 2.39  | 30 seconds                                       | 212         | 236        | 24                  |
|               | 3.12  | 30 seconds                                       | 184         | 208        | 24                  |
|               | 3.15  | 30 seconds                                       | 196         | 216        | 20                  |
| April 7.....  | 2.50  | 30 seconds                                       | 200         | 236        | 36                  |
|               | 2.52  | 30 seconds                                       | 200         | 224        | 24                  |
|               | 2.56  | 30 seconds                                       | 200         | 244        | 44                  |
|               | 3.01  | 30 seconds                                       | 200         | 240        | 40                  |
|               | 3.05  | Cerebrum removed                                 |             |            |                     |
|               | 3.19  | 30 seconds                                       | 208         | 236        | 28                  |
| April 21..... | 1.01  | 45 seconds                                       | 144         | 180        | 36                  |
|               | 1.05  | 55 seconds                                       | 152         | 184        | 32                  |
|               | 1.14  | 55 seconds                                       | 156         | 180        | 24                  |
|               | 1.20  | 55 seconds                                       | 156         | 180        | 24                  |
|               | 1.33  | 50 seconds                                       | 162         | 192        | 30                  |
|               | 1.54  | Right adrenal tied, left splanchnic cut          |             |            |                     |
|               | 2.06  | 45 seconds                                       | 164         | 164        | 0                   |
| May 7.....    | 10.35 | 30 seconds                                       | 156         | 180        | 24                  |
|               | 10.43 | 40 seconds                                       | 156         | 180        | 24                  |
|               | 10.47 | 45 seconds                                       | 156         | 180        | 24                  |
|               | 11.03 | Both adrenals removed, 100 cc. gum-salt solution |             |            |                     |
|               | 11.07 | 40 seconds                                       | 174         | 174        | 0                   |
|               | 11.10 | 40 seconds                                       | 138         | 138        | 0                   |

between 0.001 and 0.005 mgm. per k. per minute—i.e., from five to twenty-five times the amount regarded by Stewart and Rogoff as the normal output. Reflex increase of the cardiac rate does not occur if the adrenal glands are removed (see fig. 1 and table 1).

*Asphyxia.* Asphyxiation of the cat with the heart completely denervated will cause a noteworthy increase in the heart rate (see fig. 2 and table 2), an effect not seen after adrenalectomy. The figures in table 2 illustrate another point mentioned in 1917, viz., that an indication of adrenal secretion may be obtained from the denervated gland if asphyxia is prolonged. In the experiment of February 24, for example, asphyxia for 20 seconds, though previously effective, caused no change after severance of the splanchnics. In that of February 27, asphyxia of 60 seconds caused no change after splanchnic section; and in that of February 28, though asphyxia of 35 seconds had been highly effective before the splanchnics were cut, thereafter asphyxia of 45 seconds increased the heart rate only 4 beats per minute, whereas asphyxia of 90 seconds caused an increase of 68 beats a minute. Similar differences are observed in the experiment of March 21. Unfortunately these observations were not checked by final proof that cutting the splanchnics completely denervated the glands, though the marked drop in pulse rate may be regarded as testimony to that conclusion. The results are in agreement, however, with evidence adduced by Czubalski (24) that asphyxia, if sufficiently prolonged, may have a direct stimulating action on the adrenal medulla, and perhaps on other chromaffine tissue as well.

In 1917 Cannon described another method of demonstrating adrenal secretion, which consists in cutting all the nerves in the gastro-intestinal mesentery, tying all the limb arteries and the carotids, and thus leaving the circulation confined chiefly to the splanchnic area which, however, is denervated (23). Under these circumstances it is not uncommon for asphyxia to cause a slight rise of pressure after an interval of 40 to 60 seconds and a very considerably greater rise as soon as respiration begins again; these results do not occur if the adrenal glands are excluded (see fig. 3).

*Emotional excitement.* The completely denervated heart can be used as an indicator of adrenal secretion in testing the influence of emotional excitement quite as well as in testing the influence of sensory stimulation and asphyxia. It is only necessary to take somewhat greater pains in order to keep animals in normal condition after operation. To denervate the heart, the stellate ganglia are first removed

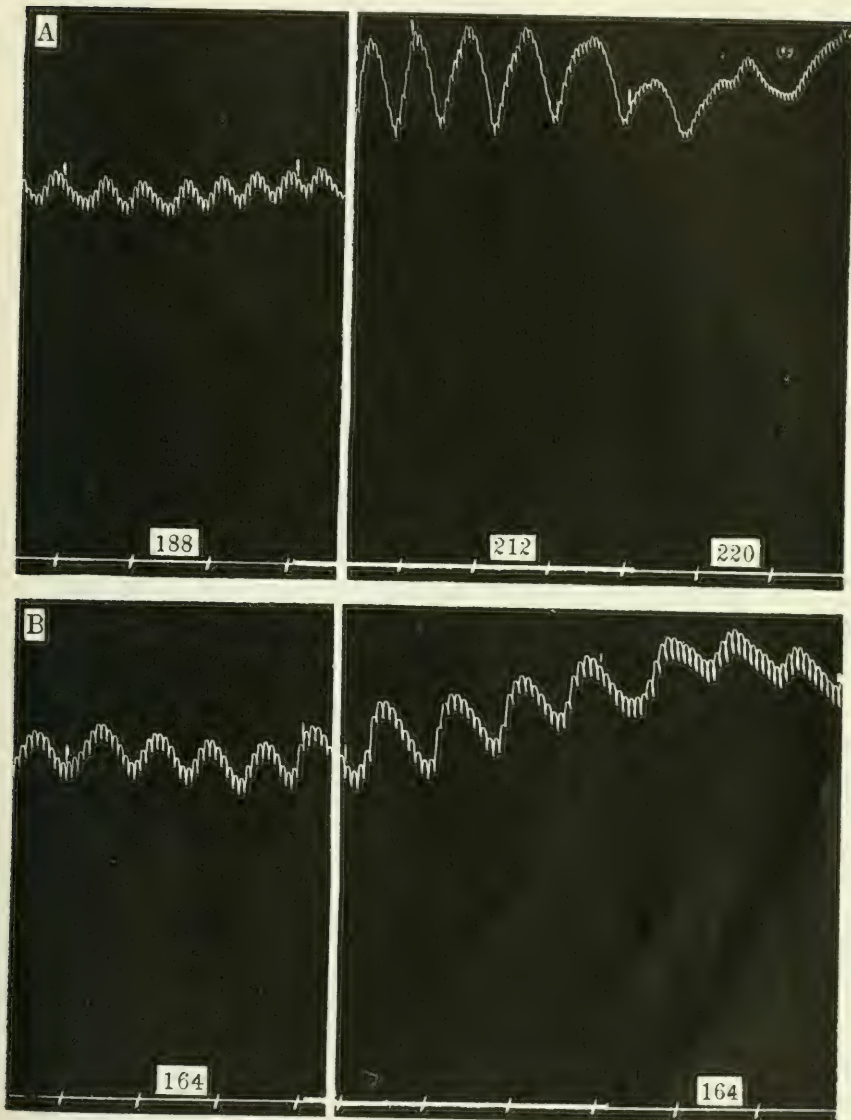


Fig. 2. Beginning and end of records of the beat of the denervated heart, mercury manometer. Original size. Time intervals, 5 seconds.

A, Asphyxia 60 seconds, 3:55. Increase of rate from 188 to 220 per minute. (Adrenal glands tied off, 4:45).

B, Asphyxia 60 seconds, 4:50. No increase of rate. Blood pressure rose from 92 to 124 mm. Hg.

TABLE 2

*Examples of increased rate of the denervated heart on asphyxiation. (In the first five cases the abdomen had been opened)*

| DATE             | TIME  | ASPHYXIA                                | RATE BEFORE | RATE AFTER | INCREASE PER MINUTE |
|------------------|-------|---|-------------|------------|---------------------|
| January 25.....  |       | 80 seconds                              | 240         | 256        | 16                  |
|                  |       | Adrenals removed                        |             |            |                     |
|                  |       | 90 seconds                              | 192         | 192        | 0                   |
| February 17..... | 2.50  | 60 seconds                              | 162         | 202        | 40                  |
|                  | 2.58  | 60 seconds                              | 158         | 200        | 42                  |
|                  | 3.01  | Veins tied both sides of adrenal glands |             |            |                     |
|                  | 3.04  | 60 seconds                              | 166         | 206        | 40                  |
|                  | 3.10  | Adrenal glands tied off completely      |             |            |                     |
|                  | 3.18  | 60 seconds                              | 146         | 146        | 0                   |
| February 24..... | 3.35  | 40 seconds                              | 204         | 256        | 52                  |
|                  | 3.38  | 20 seconds                              | 216         | 228        | 12                  |
|                  | 4.10  | Splanchnics cut in thorax               |             |            |                     |
|                  | 4.15  | 20 seconds                              | 204         | 204        | 0                   |
| February 27..... | 3.28  | 90 seconds                              | 208         | 236        | 28                  |
|                  | 3.41  | Splanchnics cut in thorax               |             |            |                     |
|                  | 3.43  | 60 seconds                              | 188         | 188        | 0                   |
| February 28..... | 11.39 | 35 seconds                              | 180         | 212        | 32                  |
|                  | 12.08 | Splanchnics cut in thorax               |             |            |                     |
|                  | 12.10 | 45 seconds                              | 164         | 168        | 4                   |
|                  | 12.13 | 90 seconds                              | 156         | 224        | 68                  |
|                  | 12.18 | 90 seconds                              | 172         | 224        | 52                  |
|                  | 12.25 | Veins tied both sides of adrenal glands |             |            |                     |
|                  | 12.31 | 90 seconds                              | 168         | 180        | 12                  |
| March 16.....    | 2.51  | 60 seconds                              | 196         | 212        | 16                  |
|                  | 3.05  | 45 seconds                              | 208         | 228        | 20                  |
|                  | 3.59  | 90 seconds                              | 188         | 224        | 36                  |
| March 21.....    | 3.09  | 45 seconds                              | 220         | 240        | 20                  |
|                  | 3.20  | Splanchnics cut in thorax               |             |            |                     |
|                  | 3.30  | 90 seconds                              | 180         | 212        | 32                  |
|                  | 3.35  | 45 seconds                              | 176         | 188        | 12                  |
| March 23.....    | 2.24  | 60 seconds                              | 192         | 236        | 44                  |
|                  | 2.34  | Abdomen opened                          |             |            |                     |
|                  | 2.53  | Adrenal glands tied off completely      |             |            |                     |
|                  | 3.20  | 60 seconds                              | 152         | 156        | 4                   |



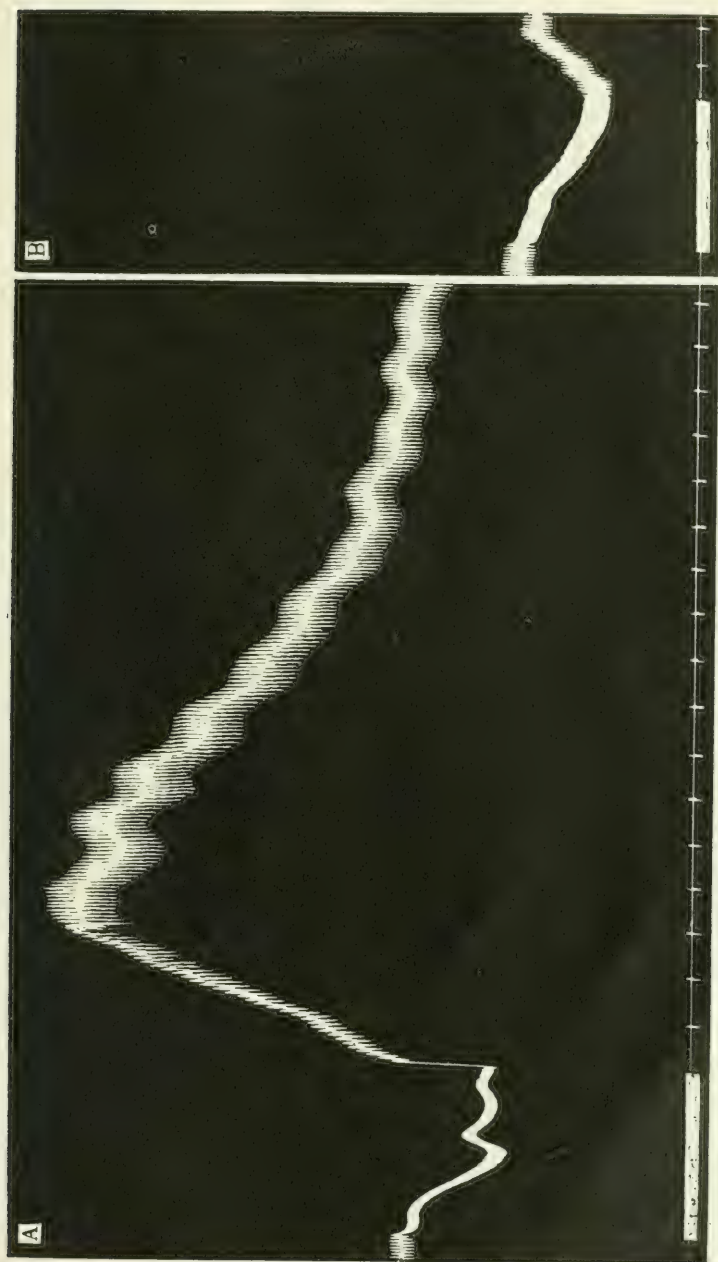


Fig. 3. Blood pressure record after tying the carotid, subclavian and iliac arteries and denervating the splanchnic area. Original size. Time intervals, 30 seconds.

A, Asphyxia 2 minutes. Rise of pressure at end of 1 minute and again after asphyxial period. (Adrenals then tied off).

B, Asphyxia 2 minutes. Both rises absent.

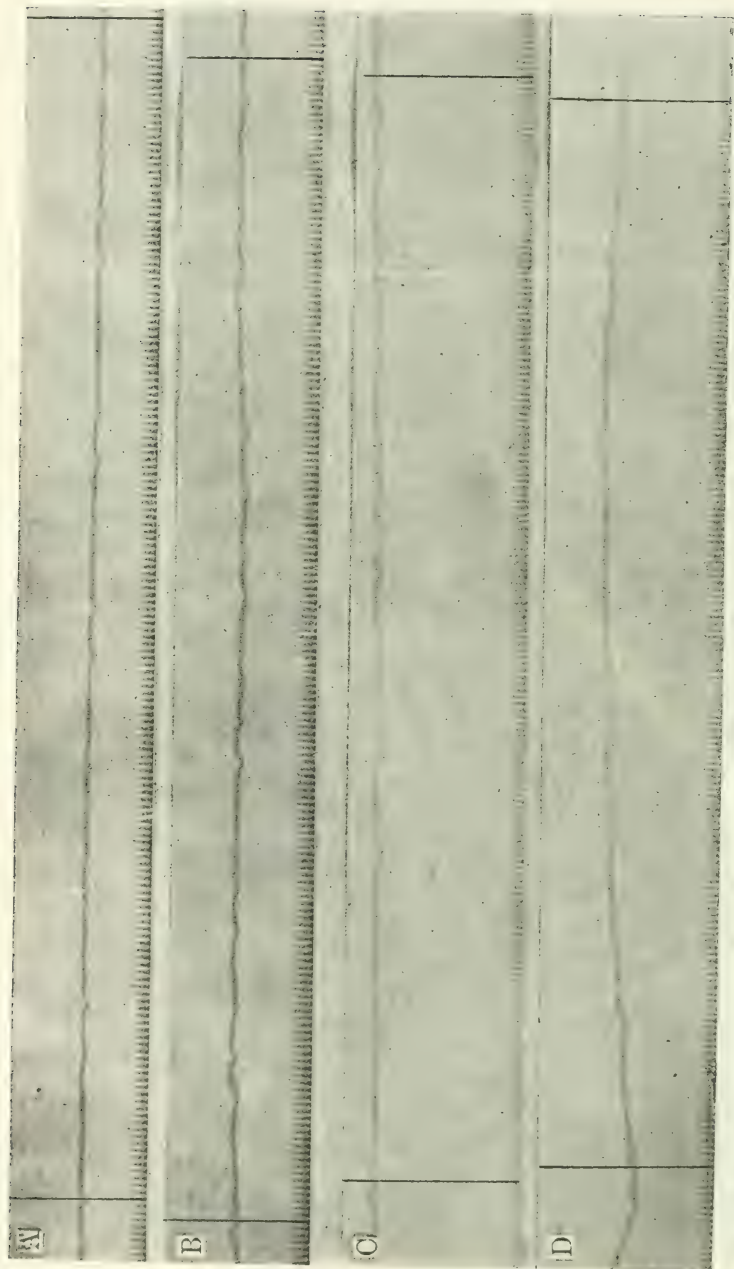


Fig. 4. Electrocardiograms of the denervated heart. Time intervals, 1/100 second. Above the time record the small dots mark 1/10 second.

A, Animal calm, heart rate 217 beats per minute. April 8.

B, Animal excited, heart rate 255 beats per minute. April 8. (Adrenal removal completed 9:50 a.m., April 10.)

C, Animal calm, heart rate 217 beats per minute. April 10, 2:40 p.m.

D, Animal excited, heart rate 221 beats per minute. April 10, 2:43 p.m.

under ether with aseptic precautions; later the right vagus nerve is severed below the recurrent laryngeal branch; and still later, the left vagus nerve is cut in the neck. The heart is thus wholly disconnected from the central nervous system and any agency causing an increase in the heart rate must exert its influence through the blood stream. In figure 4 are presented electrocardiographic records of the heart rate in a cat, operated upon as above described. The records show that with the adrenal glands normally innervated the rate was 217 per minute when the animal was calm, and 255 when excited. And after the adrenal glands were removed the rate when calm was 217 and when excited was 221.

The results obtained with the isolated heart used as an indicator of adrenal secretion thus confirm in every respect the results obtained eight years ago by the catheter method.

*Care in assuring isolation of the adrenal glands.* If the splanchnic nerves are severed or if the adrenal gland is removed on one side and the splanchnic fibers are cut on the other, as Stewart and Rogoff have noted, adrenal secretion may be isolated from nervous control in most cases, but there is not absolute certainty that this procedure will wholly eliminate nervous influences (29). For example, in one case, after the heart was wholly denervated, sciatic stimulation for one minute increased the rate from 220 to 264 beats per minute. The splanchnic nerves were then isolated in the thorax and cut. In two minutes the heart rate had dropped down to 192 beats per minute. Sciatic stimulation now increased the rate to 204, i.e., a rise of 12 beats per minute.

Similar observations have been made on animals with denervated heart that have been kept alive and observed under excitement. In one such case there was an increase of 42 beats per minute, although the left adrenal gland had been removed and the right splanchnic cut in the abdomen on the previous day. After removal of the right gland excitement had no effect. In another instance in which a similar operation had been performed there was an increase of approximately 28 beats a minute during excitement, an increase which disappeared as soon as the remaining adrenal gland was excised and the animal allowed to recover from etherization. It is possible, therefore, that other fibers than those contained in the splanchnic supply, or that occasionally, perhaps, a crossing of fibers from one splanchnic supply to the gland of the other side of the body, may be present in the cat and may thus lead to erroneous conclusions.

It has been assumed that by tying the adrenal veins at their junction with the inferior cava and the lumbar veins as they approach the adrenal glands, all possibility of an entrance of adrenal secretion into the blood stream has been excluded (10). That this may be a reasonable assumption in most cases was shown by Flint's studies of the blood supply of the cortex and medulla, which brought out the fact that the vessels of the two parts of the gland are separate. He reported, however, that as a variation from the usual condition, anastomosis may be present between the branches of the venous tree in the adrenal medulla and the venous plexus of the capsule (25). Under these circumstances the blood might flow from the medulla to the venous plexus which normally empties into the renal, phrenic and lumbar veins, into the *venae comites* of the suprarenal arteries, and to a less degree into the other veins. Further evidence of a direct vascular connection between the suprarenal gland and the veins of the kidney has been reported by Cow (26) who obtained an adrenal-like effect with blood taken from the kidney capsule of a cat.

In several instances in the course of the observations reported in this paper adrenal effects were seen after tying the lumbo-adrenal veins on both sides of the glands. In one case, after these veins had been thus tied, asphyxia caused the heart rate to increase from 166 to 206 beats a minute. The glands were then tied off completely, whereupon asphyxia had no effect (see table 2, February 17). In another instance, after the lumbo-adrenal veins had been carefully tied, injection of adrenalin into the vein as it crossed the gland caused a high rise of blood pressure, and in still another instance in which the veins were tied, splanchnic stimulation for 30 seconds caused the heart rate to rise from 172 to 248 beats per minute.

From these observations it is clear that conclusions based on results obtained when only the lumbo-adrenal veins are tied may lead to erroneous conclusions. The only absolutely safe method is that of excluding the glands from any possible action in the body by removing them or completely tying them off.

*The gradual rise of pulse rate on repeated stimulation.* A fact commonly noted in the course of the present experimentation was a gradual rise of the pulse rate with the lapse of time and with repeated indirect stimulation of the adrenal glands. In one instance an animal anesthetized with urethane had, after denervation of the heart, a pulse of 176 beats per minute. Repeated sciatic stimulation and asphyxia were accompanied by temporary increases of the pulse above the basal rate,



varying from 16 to 52 beats per minute. As these stimulations recurred, however, the basal rate gradually rose from 176 to 204. On tying off the adrenal glands completely, the rate fell to 152.

The increase of rate and its persistence at a progressively higher level with repeated stimulation are possibly facts of considerable importance in relation to the interaction of the endocrine glands, and deserve further examination.

*A fall of pulse rate on sciatic stimulation.* A curious fact noted in a number of instances after the abdomen had been opened was that sciatic stimulation, instead of causing an increase in the rate of the denervated heart, actually resulted in a slower beat. In one such case sciatic stimulation for 30 seconds reduced the rate from 216 to 212 beats per minute; subsequent stimulation for 45 seconds lowered it from 216 to 204, and still later from 236 to 216. In another instance sciatic stimulation lowered the rate from 216 to 212 and later from 232 to 212. No important changes of blood pressure preceded the altered rate. The significance of these effects is difficult to perceive. In the cases mentioned asphyxia caused a marked increase in the heart rate.

#### DEFENSE OF THE ISOLATED HEART AS AN INDICATOR OF ADRENAL SECRETION

In a recent paper on hyperglycemia, Stewart and Rogoff have incidentally offered four different arguments opposed to the conclusion that effects seen in the denervated heart are satisfactory proof of increased adrenal secretion (27). These arguments are as follows:

1. They state that there is nothing strange about an increase in the rate of the denervated heart when the central end of the sciatic or the peripheral end of the splanchnic nerve is stimulated—"it is obviously dependent upon the better blood flow through the coronary vessels." For evidence they cite Guthrie and Pike as having shown that in the perfused mammalian heart the rate could be made decidedly faster by raising the pressure of the perfusion fluid. In the experiments cited, however, Guthrie and Pike were using the *excised* heart; they definitely declare that the denervated heart *in situ* (the preparation described in this paper) does not follow the law of the excised heart as regards pressure changes. After complete denervation of the heart, they report, "there is either no change in the pulse rate (with variation of pressure), or an increase in rate with a fall in pressure, or a decrease in rate with rise in pressure." In so far as these observations testify that variations of arte-

rial pressure have *no effect* on the rate of the denervated heart, they are in accord with the earlier observations of Martin (28) and the more recent studies of Knowlton and Starling (29) who found that, between 20 and 200 mm. Hg., pressure changes did not change the rate. Frequently in the course of the work here reported arterial pressure has been raised 30 to 40 mm. Hg., after adrenal influence had been excluded, with no increase whatever in the rate of cardiac pulsation (*cf.* fig. 2). This concordant evidence wholly contradicts the first argument which Stewart and Rogoff have offered to account for the faster rate of the denervated heart when used as an indicator of adrenal secretion.

2. The second argument offered by them is that the rise of blood pressure, by increasing the rate of blood flow through the denervated heart or other organ, increases the amount of adrenin passing in unit time, and the sensitive denervated area responds to increase in the amount even if no change takes place in the rate of adrenal secretion. In presenting this argument the critics have not considered that with a rise of pressure the blood would pass more rapidly through the adrenal vessels (see p. 406); and therefore, on the basis of their own views of unvarying adrenal secretion, the higher the pressure the more diluted the adrenin—a condition which renders their argument unsound.

It is not necessary, however, to rely on argument. The simple experiment of preventing a rise of pressure may be tried. In figure 5 a record is presented of an increase in rate of the denervated heart from 144 to 180, i.e., a rise of 36 beats per minute, as a consequence of sciatic stimulation. The pressure rose about 58 mm. Hg. When the rate had fallen to 148 per minute the sciatic was again stimulated, but the pressure was prevented from rising by compression of the flexible thorax between the fingers and thumb. This is a procedure which, in the absence of the adrenal glands, is not attended by a faster beat of the denervated heart. The rate increased, however, from 148 to 184 beats per minute, a rise of 36 beats, as before. The more rapid rate developed during stimulation cannot be due to more adrenin contained in a larger volume-flow through the coronary arteries, for during stimulation the arterial pressure was not allowed to rise and augment the coronary flow. Furthermore, when the pressure was allowed to rise (50 mm.) the rapid rate, developed when the pressure was held down, did not become more rapid. The only explanation which affords a reasonable account of the faster rate is that there is something delivered to the heart through the blood stream which excites it to greater speed. Adrenin will do this. The fact that the faster rate disappears after

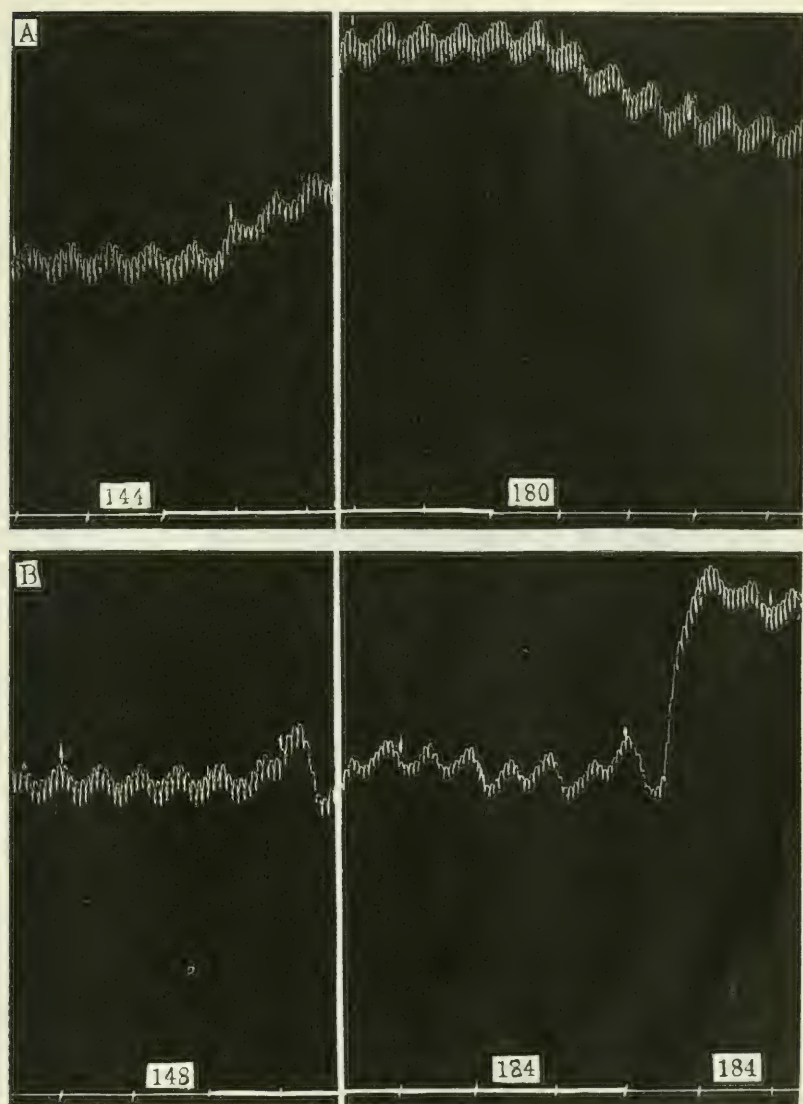


Fig. 5. Beginning and end of records of the beat of the denervated heart, mercury manometer. Original size. Time intervals, 5 seconds.

A, Sciatic stimulation 45 seconds, 1:01. Increase of rate from 144 to 180 per minute.

B, Sciatic stimulation 55 seconds (1:05). Increase of rate from 148 to 184 though pressure-rise checked by thoracic compression. No further increase with rise of pressure.

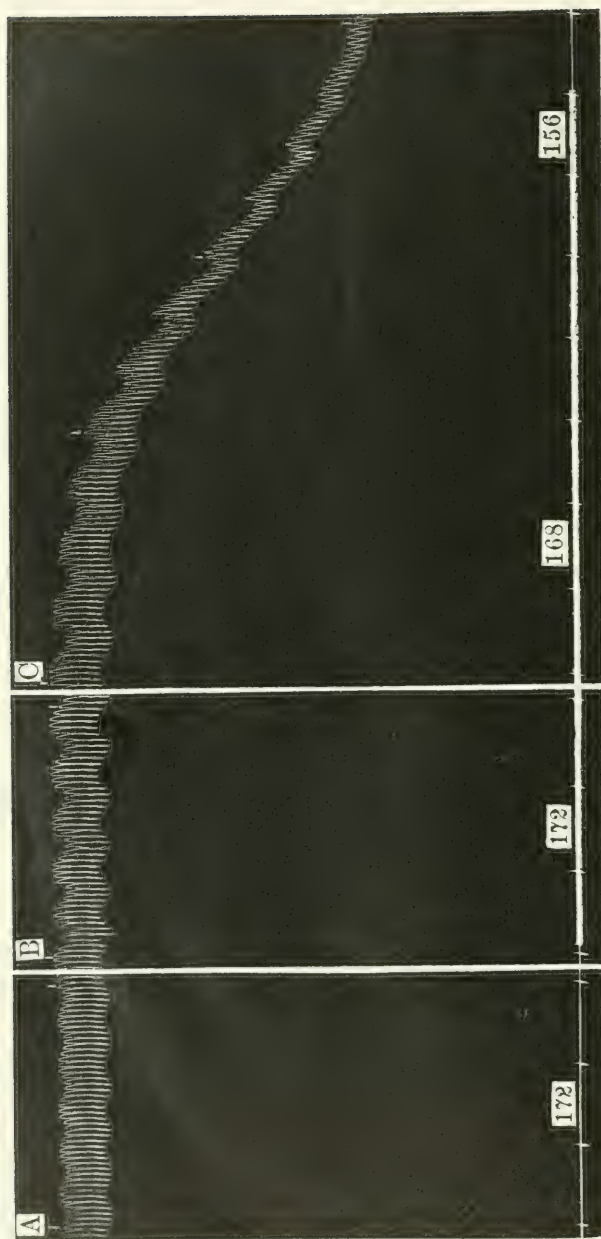


Fig. 6. Record of the beat of the denervated heart, (mercury manometer), after adrenalectomy and with a continuous uniform intravenous injection of adrenalin, 0.08 mgm. per minute. Heart rate thus increased from 132 to 172 beats per minute.

A, Time intervals, 5 seconds.

B, Beginning of 85 seconds of asphyxia. Heart rate 172 beats per minute.

C, End of 85 seconds of asphyxia. Heart rate before pressure fall, 168, later 156 beats per minute.



removal of the adrenal glands, although the rise of pressure still follows splanchnic stimulation (see fig. 1), is proof that this is the agent which is acting. Experimental test, therefore, denies the validity of Stewart and Rogoff's second argument.

3. Their third argument is directed against the use of any organ in the body as an indicator of adrenal secretion when asphyxia is employed as a stimulus, because asphyxia may be expected to alter the reactivity of the test object to adrenin, making it, for example, more sensitive. "We never supposed," they declare, "that it was possible to use in one observation an asphyxiated test object and in the comparison observation the same object with unobstructed respiration, or to assume that if there was any difference in reactions, it must be due to a difference in the rate of output of epinephrin; the condition of the test object itself being of no moment." Again this is argument and not experiment. Experiment has shown that increase of carbon dioxide causes a decrease, not an increase, in the rate of the denervated heart, and that nevertheless, adrenin, if superadded, produces a faster beat (30).

As shown in figure 6, when, after removal of the adrenal glands, adrenalin (1:200,000) is allowed to run (1 cc. in 15 seconds) steadily into a vein, asphyxia does not cause an increase of rate. The stream of adrenalin raised the rate from 132 to 172; after asphyxia had prevailed for 50 seconds the rate dropped to 168; and as the asphyxial state continued the rate became slower, dropping to 156 with a fall of pressure. A higher rate was possible, for the heart was obviously not beating at top speed, and yet there was no increase of rate at any stage in the development of asphyxia. Clearly the asphyxial condition did not render the test object more sensitive to the steady inflow of adrenalin. In the experiment illustrated in figure 2, an asphyxia lasting one minute caused an increase in the rate of the denervated heart of 32 beats a minute when the adrenal glands were connected with the circulation, but when these glands were completely tied off asphyxia for the same length of time caused no increase. The test object was in both cases subjected to identical periods of asphyxiation. Since asphyxia in the absence of the adrenal glands had no effect on the rate, whereas asphyxia with the adrenal glands present caused the characteristic acceleration which attends adrenal activity, the conclusion is warranted that the differential element in the complex, namely, the possibility of adrenal secretion, is the occasion for the typical adrenal effect. It should be remembered that Anrep (11) likewise obtained no effect of asphyxia alone,

i.e., no contraction of the denervated limb, in the absence of the adrenal glands; indeed, toward the end of the asphyxial period there was dilatation of the vessels ascribable to the direct action of the asphyxial blood on the vessel walls. This was in marked contrast to the asphyxial effect seen when the adrenal gland was present; then even a large rise of general arterial pressure, more than 50 mm. Hg., was insufficient to distend the vessels of the denervated limb, which were held contracted, according to Anrep's evidence, by secreted and circulating adrenin. Stewart and Rogoff's third argument, therefore, has no experimental warrant.

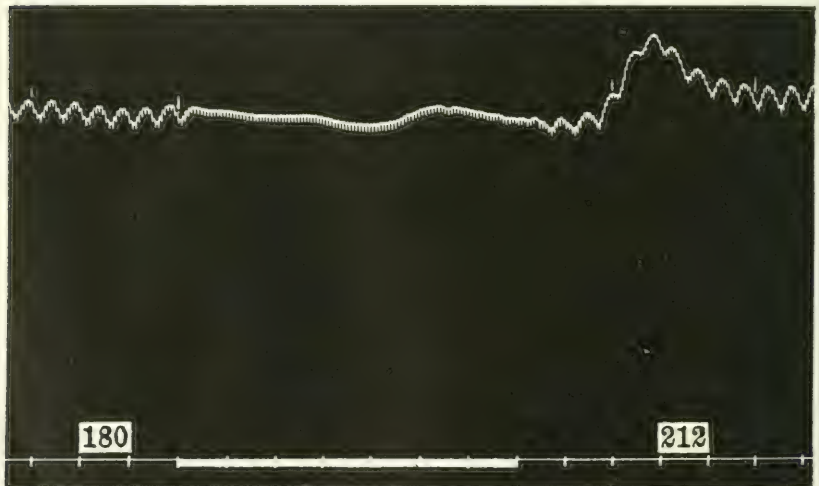


Fig. 7. Record of the beat of the denervated heart, (mercury manometer) in an animal with limb and carotid arteries tied and all mesenteric nerves severed. Enlarged one-sixth. Time intervals, 5 seconds.

Asphyxia for 35 seconds increased the heart rate from 180 to 212 beats per minute, with no noteworthy previous change in blood pressure.

4. Their fourth argument is that afferent stimulation by constricting the splanchnic vessels lessens the blood flow through the liver; in consequence the adrenal secretion contained in the cava blood is less diluted (i.e., more concentrated) than normal and therefore has more stimulating power. Again it is not necessary to rely on argument. In figure 7 is presented a record of the beats of a denervated heart in an animal in which all the nerves of the mesentery were entirely severed and the animal then asphyxiated. The rate before asphyxia was 180

beats per minute. This was increased by asphyxia 32 beats per minute. There is no possibility under these circumstances of any greater concentration of secreted adrenin because of failure of blood to pass through a constricted splanchnic area, for the nerves which would cause constriction of these vessels had been previously cut. Furthermore, the pressure did not fall, i.e., the flow was not made slower during the asphyxiated period. The effect must be ascribed to greater concentration of adrenin in blood delivered to the heart, due to an increased secretion of the adrenal medulla. The fourth argument, therefore, like the first three, fails to stand experimental test.

An observation having an important bearing on all four arguments and, indeed, on all conclusions arising from use of the "cava pocket" is that reported above (see p. 415) in giving evidence of adrenal activity at times of emotional excitement. As figure 4 shows, the rate of the denervated heart in an animal resting quietly with the adrenal glands intact was 217 beats a minute. When these glands were removed, *there was not at any time a reduction of the rate*. Recently Stewart and Rogoff (30) have testified that the "steady spontaneous discharge" from the adrenal glands in their experiments—an amount estimated as not more than 0.0002 mgm. per k. per minute—is sufficient to affect the heart. If with nerves intact there were in natural conditions the constant secretion which they declare to be "normal," removal of the glands should have been followed by a slower pulse. That the pulse did not fall below the "quiet" rate after adrenalectomy obviously permits the inference that in calm and peaceful existence there is no secretion from the adrenal glands sufficient to influence the response of an extremely sensitive indicator. In that case any attempt to explain the increased heart rate by greater delivery of adrenin or by greater concentration of it in the blood, due solely to shifts of the circulation, would be not at all pertinent.

The only factor which Knowlton and Starling found effective in causing prompt alteration of rate of the isolated heart was change of temperature. In order to increase the rate 40 beats per minute, however, the temperature of blood entering the heart had to be raised about 7°C. (29). It is inconceivable that the effects recorded above are due to the delivery of warmer blood to the heart.

From the foregoing facts the conclusion is warranted that the explanations offered by Stewart and Rogoff to account for adrenal effects on the basis of greater flow or altered distribution of the blood have no experimental support.



## CRITICISM OF METHODS YIELDING NEGATIVE EVIDENCE

A review of the previous sections of this paper reveals unanimous agreement among investigators, with the exception of Stewart and Rogoff, that painful stimulation, asphyxia and emotional excitement evoke adrenal secretion. Nevertheless, the care with which Stewart and Rogoff conducted their experiments, the quantitative methods which they employed and the variety of their experiments have led to their results being given a considerable degree of credence. As previously stated, the discrepancy between their conclusions and those reached by all other investigators naturally raises the question as to whether some difference in the methods employed would not account for the difference in the results. Since Stewart and Rogoff are alone in their contentions, it is perhaps reasonable to inquire whether the peculiar method which they employed, rather than the various methods used by others, may not have features which would account for the discrepant results.

*The method of Stewart and Rogoff.* Stewart and Rogoff obtained evidence of adrenal secretion by the use of a "pocket" in the inferior vena cava (32). This pocket was made by clamping the vena cava immediately above the iliacs, then clamping the renal veins, emptying the cava segment by stripping it upwards, and placing a clamp on the vessel above the entrance of the lumbo-adrenal veins. Any small branches of the cava segment were tied. The pocket thus formed was allowed to fill with blood from the adrenal veins, and the blood was either allowed to pass into the general circulation by removal of the clamp on the inferior cava, or was withdrawn and tested outside the body on preparations of rabbit uterus and intestine. The arrangement was modified in the "permanent pocket" by tying splanchnic vessels and shutting off the blood flow in the hind quarters. Experiments performed under these conditions revealed a spontaneous liberation of adrenin.

In one of their early papers Stewart and Rogoff state (32) that they are "not able to decide definitely whether this liberation is a normal physiological process merely unveiled by the experiments, or an abnormal process dependent upon the necessary conditions of the observations,—anesthesia, unavoidable excitation of afferent nerves, etc." They mention, however, the relative constancy of the amount secreted as in favor of the former hypothesis. Later they suggest that the extensive operation required by their procedure may have produced so



great a spontaneous discharge that no detectable increase could be produced, and they admit that Tscheboksaroff's failure to obtain increased adrenal secretion on sensory stimulation may have been due to the severe operative procedure which he employed (33). This earlier caution regarding their method they seem to have gradually abandoned, for later they mention (7) the spontaneous secretion as being the "normal output of epinephrin" and state (34) that after section of the spinal cord the secretion has all the characters of "normal secretion," and they repeatedly allude (9) to the amounts of adrenin found in the pocket as being the normal amounts. In recent papers (27), (35) they refer to their results as constituting "a striking illustration of the fact dwelt upon in previous papers that the output of epinephrin is relatively stable and not easily influenced experimentally," and they speak of the secretion occurring at a relatively constant rate as the "naturally secreted epinephrin of the organism." Rogoff (36) goes so far as to declare "it has been established beyond doubt that the adrenal glands continuously secrete a certain normal amount of epinephrin."

Before this view can be admitted, the effect of opening the abdominal cavity, clamping off the inferior cava, and repeatedly manipulating the abdominal contents, either in pressing blood out of the inferior cava or withdrawing it by syringe, must be examined. Fully twenty years ago Bayliss and Starling called attention to the profound effect which opening the abdominal cavity has on the intestines in causing them to become absolutely motionless. Local stimulation then provokes no response or only local contraction. *If both splanchnic nerves are divided*, the intestines within a short time commence to contract rhythmically and show the usual local reflexes. In order to study intestinal movements with the abdomen opened, they had to section both splanchnic nerves, or destroy the spinal cord, or excise the abdominal ganglia. "These facts," they state (37), "suggest that in the intact animal, at any rate under the conditions of our experiment, tonic or reflex influences are continually descending the splanchnic nerves and inhibiting the activity of the intestines." The observations of Bayliss and Starling may be confirmed by any one who will study gastrointestinal movements in the opened abdomen. Even if there is slight indication of activity at any time with the splanchnics intact, the least stimulation applied to the intestine, even a gentle handling of the gut, suffices to produce a reflex inhibition of its entire extent. These well-established facts make an interesting commentary on the use of the

cava pocket as a mode of obtaining evidence of "normal" or "natural" secretion. There is no doubt that secretion from the adrenal medulla is subject to impulses delivered by the splanchnic nerves, and there is no doubt that opening the abdominal cavity under anesthesia results in a discharge of impulses along these nerves. The adrenal glands, therefore, are continuously and abnormally stimulated if the abdomen is opened. The conclusion that must be drawn is that the pocket method is incapable of yielding any reliable evidence regarding the "normal" secretion of these glands.

The isolated heart yields pertinent testimony as to the discharge of impulses along splanchnic pathways under experimental conditions. An examination of the cases summarized in tables 1 and 2 reveals that, after section of the splanchnic nerves or exclusion of the adrenal glands, there is a drop in the heart rate—in some instances 40, 44 and even 48 beats per minute. The most reasonable explanation for this result is that in these experiments splanchnic impulses were continuously stimulating the glands to activity and thus making the heart beat faster than it otherwise would. Quite apart from these effects, evidence exists in the inhibitory influence of anesthesia on gastrointestinal movements that anesthesia alone can arouse splanchnic impulses (*cf.* also Elliott, *loc. cit.*). Thus the "steady spontaneous discharge" from the adrenal glands, described by Stewart and Rogoff as "normal," is confirmed and explained. But one needs only to compare the drop in heart rate after adrenalectomy in acute experimental conditions (see figs. 1 and 2) with the absence of a drop after adrenalectomy in the non-anesthetized animal (see fig. 4) to realize how abnormal is the so-called "normal" secretion which occurs during operation.

Stewart and Rogoff, after considering the possibility that their "extensive operation" may have caused so great a secretion of the adrenal glands that asphyxia, for example, could not evoke a detectable increase, became convinced that this suspicion was not well founded because they noted, on stimulating the cut splanchnic nerve directly, evidence of a decidedly greater rate of secretion (38). Obviously, when a nerve is cut and then stimulated, an unusual effect may be due to liberation of material accumulated during the inactivity which followed denervation. Furthermore, because direct stimulation of a nerve, or central excitation by strychnine, will produce certain results, that is not proof that reflex stimulation, done under anesthesia, should produce the same results. For example, there is a marked difference between the intensity of muscular response caused by direct stimulation of the

sciatic nerve and that which may be induced by reflex stimulation. Again, an abdominal operation which arouses continuous activity in the splanchnic nerves might readily interfere with splanchnic reflexes. One of the methods employed by Cannon for recording graphically the effect of secreted adrenin in the body was that of denervating the mesentery, as described above (see p. 410). This method required opening the abdomen. It yielded constant results so far as the belated influence of asphyxia was concerned, but was commonly disappointing as a means of demonstrating the early influence of asphyxia; *and in the entire series of cases with opened abdomen there was only one in which sensory stimulation caused any effect ascribable to adrenal secretion.* For example, in the first five cases of table 2, the abdomen had been opened, and in these instances, though asphyxia was effective, sciatic stimulation yielded no response whatever. From this evidence it is clear that, either because the opening of the abdomen produces a secretion unsurpassable by reflex stimulation, or because that operation abolishes abdominal reflexes, the influence of sensory stimulation on the adrenal glands is not manifested. There is little wonder, therefore, that Stewart and Rogoff, who alone have employed the pocket method, with its attendant severe abdominal operation and repeated manipulation of the abdominal contents, failed to obtain the positive results which have been obtained by all other observers.

The foregoing facts and considerations warrant the conclusion that although the work of Stewart and Rogoff was admirably quantitative in character, it was done under experimental conditions which could not afford information regarding the normal secretion of the adrenal glands or the natural conditions which affect that secretion. This conclusion applies to all inferences as to the nature of adrenal activity which they have based upon employment of the pocket method.

*The method of Gley and Quinquaud.* In the paper by Gley and Quinquaud previously mentioned (10), evidence is adduced to prove that adrenal secretion has nothing to do with the efficacy of sympathetic nervous impulses as they affect the smooth muscles of blood vessels, a conclusion well supported by the previous observations of Hoskins and McClure (39). Gley and Quinquaud removed blood from the inferior cava immediately above the opening of the subhepatic veins, and again from the right or left ventricle, in each case after splanchnic stimulation. The blood thus obtained was injected in 20 cc. amounts into other dogs weighing from 4 to nearly 10 kilos. Only the blood which was taken from directly above the opening of the



adrenal veins caused any rise of pressure in the dog injected. They conclude, therefore, that the adrenin present in adrenal blood after splanchnic stimulation is found neither in the blood of the vena cava above the subhepatic veins nor in the blood of the heart.

In drawing this conclusion Gley and Quinquaud seem to have disregarded the fact that they were, in the first place, taking only a small portion of the secreted adrenin, which had already been diluted by the blood of the donor, and were then injecting this small portion into the blood stream of another dog, where it would be diluted to a much greater degree.

Gley and Quinquaud declare categorically that secreted adrenin is not carried by the circulation to the organs on which it acts, and that, if present at all, it is present in a quantity altogether minimal and insufficient to exercise its action. This declaration again is made without due regard to evidence already in the literature. The observations on the denervated limb, on the denervated kidney, on the denervated salivary gland and on the denervated heart, quoted or described above, clearly demonstrate that adrenal secretion may be stimulated by painful impulses, by asphyxia and by emotional excitement, and that the substance secreted under these circumstances not only is carried to the structures on which it acts, but produces on these structures pronounced physiological effects. Until this evidence is definitely proved to be unworthy of acceptance, the conclusion which Gley and Quinquaud have drawn must be regarded as quite unjustified.

#### INTERPRETATION OF THE FUNCTION OF THE ADRENAL MEDULLA

With the disappearance of the view that the adrenal glands produce some substance which neutralizes toxic material developed in the body, there have been left two theories to account for the rôle played by the adrenal medulla in the bodily economy. These are the tonus theory and the emergency theory.

The tonus theory, which has been advocated in the past (40) and still receives attention, holds that the function of the secreted adrenin is to maintain the sympathetic endings in a state of responsiveness to nervous stimulation or in a condition of moderate activity or tone. This view has definitely lost ground in the course of relatively recent investigations. A number of investigators have called attention to the depressive effect of small doses of adrenalin (39), (41). If the smallest dose which will have any influence whatever on the blood



vessels induces relaxation of the vessels, it is difficult to understand how the function of the secreted adrenin could be that of maintaining a state of tonic contraction. Furthermore, as has been repeatedly noted (42), double adrenalectomy does not for some time cause the fall of arterial pressure which naturally would be expected if continued adrenin secretion were needed to keep the pressure up; and also stimulation of the splanchnic nerves induces the same rise of pressure after adrenalectomy as before (10). From these results the conclusion has been drawn by Hoskins and McClure and by Gley and Quinquaud that the tonus theory is without adequate experimental support.

The emergency theory was presented by Cannon on the basis of studies of adrenal secretion following stimulation of afferent nerves, asphyxia and emotional excitement. In the papers bearing upon this theory emphasis was repeatedly laid upon the association between adrenal activity and the activity of the sympathetic division of the autonomic system in such emergencies. Nowhere has the statement been made that secreted adrenin has a function separate from that of the nerve impulses, except to increase the irritability of fatigued muscles (3) and to speed the coagulation of the blood (4). The idea originally suggesting these studies on adrenal secretion was that changes in the viscera originally induced by nervous impulses might be continued by circulating adrenin (43, p. 40). No claim has ever been made that there is at any stage a primacy of adrenin in the production of physiological or psychological changes seen during strong emotion.

In spite of the foregoing facts authors have written as if Cannon had been attempting to support the idea that emotional experiences were dependent upon circulating adrenin. Thus Stewart and Rogoff report (32), as if the matter had been questioned, that all signs of fright can be elicited by administering morphine to a cat with one adrenal removed and the other denervated.<sup>6</sup> Rogoff points out (36) that the secretion of sufficient adrenin to produce symptoms of fright would be impossible, again as if any claim had been made that these symptoms were due to secreted adrenin. Gley and Quinquaud declare that the

<sup>6</sup> Stewart and Rogoff noted dilatation of the pupil of the "denervated eye" when animals became frightened, though one adrenal was removed and the nerves to the other sectioned. Cannon and de la Paz tried this method of testing for adrenal secretion but could not persuade themselves that an eye still innervated by the third cranial nerve was really "denervated" and interpreted the prompt dilatation of the pupil in a paroxysm of rage as due to central inhibition of the still active constrictor muscles (see Cannon: *Bodily Changes in Pain, Hunger, Fear and Rage*, New York, 1915, p. 35).

persistent irritability of the nerves after the adrenal glands have been removed is opposed to the explanation which Cannon has given to experiments on the adrenin origin of emotions (10). Indeed, Cannon has been definitely charged with assuming that the reaction to fear and other emotional states is dependent on hypersecretion of adrenin (44). Careful reading of his work gives no support for these interpretations.

The concept of an emotion may be expressed either in psychological terms of subjective experience or in physiological terms of bodily change. Cannon's observations lend no support to the idea that adrenal secretion is essential to the subjective experience of strong emotion. Adrenin has its effect peripherally, on outlying viscera. An assumption that subjective feeling depends on circulating adrenin involves, therefore, supporting the view that emotion as a psychological state is the consequence of visceral changes. Cannon has, in fact, definitely argued against this view (43, p. 275).

If the critics of the emergency theory conceive emotion as bodily change, they will find in Cannon's consideration of the interrelations of emotions the point emphasized that it is the *sympathetic division of the autonomic system* which is the primary agency in mobilizing the bodily forces in times of great fear or rage (43, p. 268). To assume that secreted adrenin is necessary for the changes which occur under such conditions implies an acceptance of the tonus theory. This view has not been held by Cannon and receives no support in any observation he has reported. The only suggestion which he has offered (43, p. 64) that might be construed into support of such a view is that adrenal secretion given forth into the blood stream during excitement is a substance capable of inducing or augmenting the nervous influences which bring about the very changes in the viscera that accompany excitement. Naturally, this suggestion should be considered in conjunction with others; e.g., "it is possible that disturbances in the realm of the sympathetic are automatically augmented and prolonged through chemical effects of the adrenal secretion" (43, p. 38), and "the changes originally induced in the digestive organs by nervous impulses might be continued by circulating adrenin" (43, p. 40). These suggestions imply coöperation of chemical and nervous factors, but not a dependence of the nervous factors on the chemical.

The possibility has been recognized (43, p. 65) that in times of emotional stress there may be coöperation of secreted adrenin with the products of other endocrine glands simultaneously excited, which might render the adrenin much more effective than it would be by itself.

This is a possibility which should be kept in mind in connection with the emergency theory of adrenal secretion. Until this possibility has been tested, however, there is no need of going further than the facts will warrant in appreciating the coöperative character of secreted adrenin and sympathetic nervous impulses.

Thus far no reliable evidence has been brought out by any investigator that there is any secretion whatever of the adrenal glands under quiet, peaceful conditions. Results reported in this paper present the first indication that under such conditions there is no adrenal secretion or a secretion so slight as not to affect the denervated heart, an extremely sensitive indicator. Stewart and Rogoff have shown that the cat and the dog will live normally for weeks with one adrenal excised and the other denervated, an operation which results in no demonstrable flow of adrenin from the adrenal vein (45). These observations prove that adrenal secretion is not a necessity, at least in times of serene existence. Adrenin is secreted, however, in times of great emotional stress and under circumstances which cause pain or asphyxia. As stated at the beginning, the function of the adrenal medulla is to be looked for under conditions which rouse it to action. Excitement, pain or asphyxia are, in natural existence, commonly associated with violent struggle for self-preservation. Under such circumstances, as has been emphasized in the presentation of the emergency theory, the operation of the sympathetic division of the autonomic system together with the aid which adrenin affords will muster the resources of the organism in such a way as to be of greatest service to such organs as are absolutely essential for combat, flight or pursuit. It appears, therefore, that the emergency theory of the adrenal medulla is the only one which thus far has any experimental support.

It is a pleasure to express my thanks to Mr. H. F. Pierce for help in the early experiments above reported, and to Dr. Alexander Forbes for making the electrocardiograms.

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# EFFECT OF WORK AND HEAT ON THE HYDROGEN ION CONCENTRATION OF THE SWEAT

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## INTRODUCTION

Early in the year 1918 my attention was attracted to some work that had been done several years ago demonstrating, incidentally, how muscular fatigue might affect the reaction of the urine. The fact that the results were conflicting, and particularly that all the investigators used methods of titration, which we now recognize as quite unreliable for obtaining total acidity, led me to think that this might prove an interesting field for research. Furthermore, inasmuch as the literature seemed to be silent as to whether exercise in any way affected the reaction of the sweat, it seemed to be desirable to make determinations on the urine and sweat simultaneously, with the subject under the same conditions, with the view first, of establishing more definitely the results of the earlier observers as far as the urine was concerned; second, to ascertain if any reaction changes occur in the perspiration; and third, whether these changes, if they occur, show either a supplementary or a compensatory relationship with the urine.

With these ideas in mind I made a few preliminary tests in Ripon College chemical laboratory through the courtesy of Dean Barber and Professor Barker. However, the problem was not attacked in an intensive manner until I had the privilege of pursuing the work during the school year of 1918-1919 in the physiological laboratory of Professor Howell in the School of Hygiene and Public Health of the Johns Hopkins University. It therefore seems most fitting that I should here acknowledge my deep gratitude to him for his helpful suggestions and above all the inspiration that I received from his kindly interest. In the same manner I wish to thank Doctor Spaeth of this laboratory for assistance with the experimental technique.

It is my purpose in this article to consider simply the perspiration, and to discuss subsequently the results obtained from the urine.

We have in the past been quite willing to assume that the principal function of the sweat glands is for the regulation of body temperature, and not much emphasis has been laid upon the significance of the composition of the excretion.

Before entering upon the discussion of the problem some of the views that have been held in the past as to the reaction of the sweat, more or less conflicting, may be stated briefly.

Foster (1) states that "Sweat from a well washed skin is alkaline. It is only when mixed with sebum that it is acid. Horses' sweat is said to be always acid." Smith (2) finds horses' sweat strongly alkaline. Moriggia (3) states that in herbivora the reaction is generally alkaline, while in carnivora it is acid. Gaube (4) speaks of the human sweat as acid, while that of the horse, cow, dog, cat and hog is alkaline. Certainly omnivora, herbivora and carnivora are found in this last list. In Landois' text (5) we find the statement that "swine sweat (?) on the snout, cattle about the mouth (?), while goats, rabbits, rats, mice and dogs do not sweat at all." Aron has stated that monkeys have no sweat glands but Shaklee (20) in later work reports that this is an error, "the skin seems everywhere provided with well-developed sweat glands."

#### METHODS

As has been suggested above, the criticism that may be made upon earlier work is concerned with the uncertainty of the data obtained by titration. In the observations which follow, use was made of the colorimetric and the gas chain methods in order to obtain the total acidity. As to the former, I followed mainly the method of Henderson and Palmer (6), which is an adaptation of the Sørensen method, while for the gas chain measurement I used a Leeds and Northrop's potentiometer with Clark's (7) hydrogen electrode shaker. It was hardly thought necessary to use a constant temperature chamber, but a thermometer was kept in the potassium chlorid vessel and therewith the corrections were made.

For the colorimetric method it may be stated briefly that there are required five standard solutions made up of monopotassium phosphate and disodium phosphate, mixed in such proportions that each solution will possess a distinct and definite pH value. In like manner, acetic acid and sodium acetate are so mixed as to give six standard solutions of

definite pH values. The former mixture is for use on the alkaline side with values ranging from 8.7 pH to about 7.0 pH. The latter reaches from 7.0 pH to 4.7 pH. These standards are as a rule made fresh once a week, but never kept longer than two weeks. When ready for tests, 4 cc. of each standard was taken and diluted with distilled water up to the mark in a 100 cc. flask, and a similar dilution was taken for the sweat. An equal amount of indicator added to the same volume of the diluted standard and the diluted sweat and the colors thereby matched gave a fair evaluation for the reaction of the latter even where interpolation was necessary.

As to the indicators, phenolphthalein can be used for values between 8.7 pH and 8.0 pH. Neutral red, however, is very useful between 8.0 pH to even below the neutral point 7.0 pH. Methyl red is excellent on the acid side from 5.7 pH to 4.7 pH, and is not affected by proteins, while p.nitrophenol covers a wider range and is an excellent indicator between 6.7 pH and 4.7 pH. Sodium alizarine sulphonate covers the entire field from 8.0 pH to 4.7 pH, thereby meeting all of the variations that I have found in the perspiration. There were, at times, in use the indicators that are recommended by Clark and Lubs (8), which were quite satisfactory.

In the main the determinations were made by the colorimetric method, while the potentiometer was used for checking up the standard solutions.

Unfortunately the gas chain was not brought into use as often as one might wish, because at times tedious delays were caused by not being able to get the services of a mechanician.

I am aware that objections may be urged against placing too much reliance upon absolute evaluation by the colorimetric method. "Off-shades" did arise at times, especially with the use of sodium alizarine sulphonate. In that case determinations had to be made with other indicators or the test would have to be rejected.

The protein and salt errors have been long recognized by Sørensen and others. As to the former, I have a feeling that the per cent in the sweat is so low that its effects are quite negligible. As to the latter, I have not that same confidence, for the work of Viale (9) shows variations in the salt content. Of late some have laid considerable emphasis upon the variations due to the carbon dioxid factor.

However, after making due allowance for these liabilities to error it must not be overlooked that Lubs and Clark (10) and others have obtained some remarkable agreements in the two methods.



Granting that there might be some slight errors in the absolute, there can hardly be any errors in the relative values, as the samples to be compared were tested almost simultaneously.

My first tests were made on volunteers from the Baltimore Central Y. M. C. A. and upon a few of the workmen about the laboratory. These were hardly more than preliminary try-outs, as was ultimately proven. In some cases the skin was cleansed with water or alcohol, while in others not at all. The samples which were collected at the Y. M. C. A. were really the combination effect of heat and work, for the subjects, after playing basket-ball or volley-ball, as the case might be, came into the hot-room where the samples were obtained.

One important point revealed by these tests was that the sweat contains a greater concentration of hydrogen ions than one would naturally suppose.

However, the futility of the volunteer plan soon became apparent. It was evident that in order to make the experiments worth while it was necessary to have reliable and well selected subjects who would be willing to come to the laboratory, where it would be possible to have better controls. This end was realized by obtaining as subjects some medical students, who had a much better appreciation of the importance of the work and were willing to coöperate in a most excellent manner.

The few subjects that I used, mainly, were in perfect health, as they had passed the tests as donors of blood for transfusion cases. After undertaking the work in the laboratory my first thought was to ascertain if exercise produced any changes in the hydrogen ion concentration of the sweat. Consequently there arose the suggestion of a control.

My idea was to obtain heat-sweat first and then secure work-sweat immediately afterwards, with the feeling that if any change took place in the latter it would be as in the case of the urine in the elimination of more acid as a result of work.

The subjects were stripped and the parts from which the sweat was to be taken, viz., face, chest and abdomen, were first washed with soap followed by cleansing with water, ether and alcohol in the order named. In the application of the last two liquids dental napkins were used. The subjects were then placed in a hospital sweat-cabinet with a fair amount of moisture, starting with a temperature of about 30°C. and finally increasing it to 40° or 45°C. The heating lasted from fifteen to twenty-five minutes, according to the subject and the number of samples to be obtained. All samples of sweat were collected by means of lipless specimen tubes.



After this procedure, the parts being controlled as before, the subject was placed on a stationary bicycle where he worked for fifteen to twenty-five minutes. The samples of work-sweat and heat-sweat were then tested as soon as possible and comparisons were made.

The most striking thing about the data, in the securing of which there were sixteen observations upon six different individuals, was that in each instance the heat-sweat was of a greater hydrogen ion concentration than the work-sweat. (See table 1, series A.)

TABLE 1

| HEAT PRECEDING WORK SERIES A |               |               |               | WORK PRECEDING HEAT SERIES B |               |               |               |
|------------------------------|---------------|---------------|---------------|------------------------------|---------------|---------------|---------------|
| Subjects                     | pH heat-sweat | pH work-sweat | Difference    | Subjects                     | pH work-sweat | pH heat-sweat | Difference    |
| G.                           | 5.4           | 5.9           | 0.5           | G.                           | 5.8           | 5.4           | 0.4           |
| G.                           | 5.5           | 5.65          | 0.15          | G.                           | 5.8           | 5.5           | 0.3           |
| G.                           | 5.25          | 5.8           | 0.55          | G.                           | 5.7           | 5.5           | 0.2           |
| J.                           | 5.8           | 6.4           | 0.4           | G.                           | 5.9           | 5.2           | 0.7           |
| J.                           | 6.4           | 6.6           | 0.2           | G.                           | 5.9           | 5.15          | 0.75          |
| J.                           | 5.4           | 7.0           | 1.6           | J.                           | 5.6           | 5.6           | 0.0           |
| E.                           | 7.2           | 7.5           | 0.3           | J.                           | 6.1           | 5.5           | 0.6           |
| E.                           | 7.1           | 7.4           | 0.3           | E.                           | 7.4           | 5.3           | 2.1           |
| E.                           | 6.0           | 7.4           | 1.4           | F.                           | 5.8           | 5.2           | 0.6           |
| E.                           | 5.6           | 7.4           | 1.8           | C.                           | 5.45          | 4.7           | 0.75          |
| E.                           | 5.7           | 6.5           | 0.8           | S.                           | 6.15          | 5.5           | 0.65          |
| E.                           | 5.7           | 6.5           | 0.8           |                              |               |               |               |
| E.                           | 5.6           | 6.8           | 1.2           | 6                            | 5.96          | 5.32          | Av. dif. 0.64 |
| B.                           | 6.2           | 7.4           | 1.2           |                              |               |               |               |
| A.                           | 5.6           | 6.0           | 0.4           |                              |               |               |               |
| M.                           | 5.1           | 5.9           | 0.8           |                              |               |               |               |
| 6                            | 5.22          | 6.63          | Av. dif. 1.41 |                              |               |               |               |

Fearing that the first excretions might be more acid due to the sebum or other causes, I reversed the process by producing work-sweat first. I made eleven observations with the use of six individuals, with the result that the heat-sweat was still of higher acidity. (Note table 1, series B.)

I next adopted the plan for a few experiments upon subject G. of producing sweat in the morning by work and by heat in the afternoon, and then reversed this process, with no particular differences in the results.

All of the remainder of the experiments were performed on two individuals simultaneously, one producing heat-sweat and the other work-

TABLE 2

| TESTS | SUBJECTS | pH<br>WORK | pH<br>HEAT | AVERAGE<br>DIFFER-<br>ENCE | TESTS | SUBJECTS | pH<br>WORK | pH<br>HEAT | AVERAGE<br>DIFFER-<br>ENCE |
|-------|----------|------------|------------|----------------------------|-------|----------|------------|------------|----------------------------|
| 1     | G.       | 5.9        | 5.5        |                            | 1     | E.       | 7.5        | 7.2        |                            |
| 2     | G.       | 5.65       | 5.4        |                            | 2     | E.       | 7.4        | 7.1        |                            |
| 3     | G.       | 5.8        | 5.25       |                            | 3     | E.       | 7.5        | 7.2        |                            |
| 4     | G.       | 5.8        | 5.5        |                            | 4     | E.       | 7.4        | 7.1        |                            |
| 5     | G.       | 5.7        | 5.2        |                            | 5     | E.       | 7.4        | 6.0        |                            |
| 6     | G.       | 5.9        | 5.0        |                            | 6     | E.       | 6.5        | 5.6        |                            |
| 7     | G.       | 5.8        | 5.25       |                            | 7     | E.       | 6.5        | 5.7        |                            |
| 8     | G.       | 5.7        | 5.5        |                            | 8     | E.       | 6.8        | 5.7        |                            |
| 9     | G.       | 5.6        | 5.4        |                            | 9     | E.       | 7.4        | 5.6        |                            |
| 10    | G.       | 5.8        | 5.4        |                            | 10    | E.       |            | 5.8        |                            |
| 11    | G.       | 5.8        | 5.4        |                            | 11    | E.       |            | 5.3        |                            |
| 12    | G.       | 6.0        | 5.85       |                            |       |          | 7.15       | 6.24       | 0.91                       |
| 13    | G.       | 5.6        | 5.6        |                            |       |          |            |            |                            |
| 14    | G.       | 5.6        | 5.6        |                            |       |          |            |            |                            |
| 15    | G.       | 5.8        | 5.4        |                            |       |          |            |            |                            |
| 16    | G.       | 5.8        |            |                            | 1     | J.       | 6.4        | 5.8        |                            |
| 17    | G.       | 5.7        |            |                            | 2     | J.       | 6.6        | 6.4        |                            |
| 18    | G.       | 5.35       |            |                            | 3     | J.       | 5.6        | 5.4        |                            |
| 19    | G.       | 5.35       |            |                            | 4     | J.       | 6.1        | 6.4        |                            |
| 20    | G.       | 5.9        |            |                            | 5     | J.       |            | 5.8        |                            |
|       |          |            |            |                            | 6     | J.       |            | 5.2        |                            |
|       |          | 5.73       | 5.42       | 0.31                       |       |          | 6.18       | 5.83       | 0.35                       |
| 1     | M.       | 5.1        | 5.1        |                            |       |          |            |            |                            |
| 2     | M.       | 5.4        | 5.4        |                            |       |          |            |            |                            |
| 3     | M.       | 5.95       | 5.85       |                            |       |          |            |            |                            |
| 4     | M.       | 5.65       | 5.6        |                            |       |          |            |            |                            |
| 5     | M.       | 5.8        | 5.6        |                            |       |          |            |            |                            |
| 6     | M.       | 5.8        | 5.4        |                            |       |          |            |            |                            |
| 7     | M.       | 6.4        | 5.8        |                            |       |          |            |            |                            |
|       |          | 5.84       | 5.54       | 0.30                       |       |          |            |            |                            |

TABLE 3

| OBSERVATION | SUBJECTS | pH WORK  | OBSERVATION | SUBJECTS | pH HEAT  |
|-------------|----------|----------|-------------|----------|----------|
| 20          | G.       | 5.73     | 15          | G.       | 5.42     |
| 7           | M.       | 5.84     | 7           | M.       | 5.54     |
| 9           | E.       | 7.15     | 11          | E.       | 6.24     |
| 4           | J.       | 6.18     | 6           | J.       | 5.83     |
| 13          | X.       | 6.19     | 14          | X.       | 5.61     |
| Total 53    |          | Av. 6.22 | Total 53    |          | Av. 5.73 |

TABLE 4

| SERIES A HEAT-SWEAT pH |              |               |                    | SERIES B WORK-SWEAT pH |              |               |
|------------------------|--------------|---------------|--------------------|------------------------|--------------|---------------|
| Subjects               | First sample | Second sample | Third sample       | Subjects               | First sample | Second sample |
| G.                     | 5.5          | 5.6           | 5.5<br>5.6<br>5.55 | G.                     | 5.7          | 5.8           |
| G.                     | 5.5          | 5.65          |                    | G.                     | 5.6          | 5.65          |
| G.                     | 5.2          | 5.45          |                    | G.                     | 5.8          | 5.9           |
| G.                     | 5.12         | 5.2           |                    | G.                     | 6.0          | 5.8           |
| G.                     | 5.0          | 5.2           |                    | G.                     | 5.6          | 5.55          |
| G.                     | 5.25         | 5.3           |                    | G.                     | 5.8          | 5.6           |
| G.                     | 5.5          | 5.55          |                    | G.                     | 5.7          | 5.9           |
| G.                     | 5.4          | 5.55          |                    | G.                     | 5.9          | 5.8           |
| G.                     | 5.85         | 5.15          |                    | G.                     | 5.35         | 5.3           |
| G.                     | 5.6          | 5.45          |                    | G.                     | 5.7          | 5.75          |
| G.                     | 5.6          | 5.3           |                    | G.                     | 5.9          | 5.95          |
| G.                     | 5.4          | 5.45          |                    | G.                     | 5.9          | 5.6           |
| G.                     | 5.4          | 5.5           |                    | G.                     | 5.65         | 5.7           |
| G.                     | 5.5          | 5.6           |                    | G.                     | 5.8          | 5.75          |
| G.                     | 5.25         | 5.2           |                    |                        |              |               |
| M.                     | 5.4          | 5.15          | 5.5                | M.                     | 5.5          | 5.8           |
| M.                     | 8.0*         | 5.2           | 5.25               | M.                     | 5.95         | 5.15          |
| M.                     | 5.85         | 5.8           |                    | M.                     | 5.65         | 5.9           |
| M.                     | 5.6          | 5.35          |                    | M.                     | 5.8          | 5.85          |
| M.                     | 5.4          | 5.25          |                    | M.                     | 5.8          | 5.4           |
| M.                     | 5.8          | 5.6           | 5.8                | M.                     | 6.4          | 6.3           |
| M.                     | 5.1          | 5.2           | 5.4                | M.                     | 5.9          | 5.8           |
|                        |              |               |                    | M.                     | 5.8          | 6.0           |
| E.                     | 5.9          | 5.7           |                    |                        |              |               |
| E.                     | 5.4          | 5.25          |                    |                        |              |               |
| F.                     | 4.85         | 4.7           | 5.1                |                        |              |               |
| S.                     | 5.35         | 5.7           | 5.8                |                        |              |               |
| J.                     | 5.2          | 5.35          | 5.3                |                        |              |               |
| O.                     | 5.9          | 5.7           |                    |                        |              |               |

\* This unusual reading may be due to the fact that the subject had served as a donor in a blood transfusion three hours before the experiment.

sweat, while the next day the performance was reversed. The grand totals from these experiments are given for four of the subjects in table 2.

Table 3 gives averages of the four principal subjects, while X. represents the average of several individuals taken together where not more than one or two tests were made on each person.

In table 4, series A, are found twenty-eight experiments in which two samples of heat-sweat were taken, while in series B of the same table we have twenty-two experiments in which two samples of work-sweat were taken. It is a remarkable coincidence that just 50 per cent of the second samples increased in acidity in both kinds of experiments.

There were ten tests in which three samples were taken successively as a result of heat. Comparing the third with the first sample we now find that eight are less acid, while one is more acid and one remains the same. In comparing the third with the second sample we again find that eight are less acid, one is more so, while one remains the same. The skin was cleansed as before after each collection, and an interval of five minutes elapsed between the collection of the successive samples.

#### CONCLUSIONS

In my conclusions I wish to emphasize that in many cases the changes were exceedingly small, yet the distinction was always obvious. Furthermore it is to be remembered that the work experiments were not long-enduring and fatiguing tests like those that have been reported in the past on the urine. On the contrary the subjects as a rule had performed their part of the experiments within one hour. While the time was short, the experiments, especially from exercise, were rather intense. In a majority of cases these tests took place late in the forenoon, ranging from two to five hours after a meal.

The work reveals the following facts:

*First*, that sweat caused from either work or heat is acid, probably always so in perfect health, and the degree of acidity is greater than we have heretofore believed.

*Second*, in a continued secretion of sweat the reaction does not remain entirely constant. A second sample may show a slight increase or decrease in acidity, while a third sample shows practically in all cases a small but distinct diminution compared with the first sample.

*Third*, the sweat caused by external heat is always more acid than that caused by muscular work.



Many authors have assumed that the secretion of sweat is normally alkaline and that the acid reaction actually shown in many cases is due to admixture with sebaceous secretion. But Franeois-Franck (11), Kittsteiner (12) and others, state that the sweat from the palm of the hand is acid, although this portion of the skin is devoid of sebaceous glands. The observations reported in this paper also throw doubt upon this explanation of the acidity of sweat as usually collected, since the precautions taken to cleanse the skin before collecting the sample should have been sufficient to remove any deposit of sebaceous material. The immediate cause of the acidity of sweat has not been determined satisfactorily. Halliburton (13) states that the sweat, like the urine, contains acid phosphates, but this explanation has not been corroborated by satisfactory analyses. Others have assumed the presence of volatile fatty acids in the secretion, and some observations of my own tend to support this view. In a number of cases the insensible perspiration was collected by fixing a finger suitably in a glass chamber, so that the vapor would condense upon the walls of the vessel. When diluted and compared with distilled water this condensate gave always an acid reaction. Aubert (14), Röhig (15), Schierbeck (16), Fubini and Ronchi (17), have emphasized the importance of carbon dioxide. The amount of carbon dioxide excreted varies with exercise and especially with external temperature. Schierbeck, for example, found that at a temperature of 29.8°C. there was an elimination of 8.9 grams of carbon dioxide in twenty-four hours, while at a temperature of 38.4°C. the amount excreted in the same period was 29.5 grams. The larger excretion of carbon dioxide would increase the acidity of the sweat as secreted, but presumably this factor did not enter into the reactions as determined by the method described in this paper. In these determinations the diluted specimens were exposed freely to atmospheric air, and presumably took on a corresponding tension of carbon dioxide.

The statement of Heuss (19), "*Der schweiss reagirt in der Ruhe normaler weise sauer bei profuser secretion (Pilocarpin, Schwitzbäder) kann er neutral ja sogar alkalisch werden,*" I could hardly support unreservedly. So far I have not tested any sweat produced by drugs, so I have nothing to offer on that point. However, when I have followed work with heat I have found that the sweat from the latter is not only more acid, but more profuse.

So the question of the profuseness, so often referred to in the literature, does not in itself offer a satisfactory explanation of differences in reaction.

The fact that heat-sweat shows uniformly a higher hydrogen ion concentration than work-sweat is surprising and contrary to expectation. In muscular work there is a large increase in the acid products of metabolism, and the output of acid in the sweat can be understood as part of the mechanism for preserving the acid-base equilibrium of the body. In heat-sweat we have heretofore regarded the secretion and evaporation of the water as an important means of controlling the body-temperature, the so-called physical regulation of the heat-equilibrium of the body. The fact that this heat-sweat is acid may be looked upon as a demonstration that the sweat in man under ordinary dietary conditions is normally acid, and that this secretion, like that of the urine, helps to maintain the acid-base equilibrium of the organism. But why the heat-sweat should exhibit a greater acidity than the work-sweat is not clear. It is hoped that further study of this problem may prove not only of physiological, but of therapeutical value.

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# EFFECT OF PHYSICAL TRAINING AND PRACTICE ON THE PULSE RATE AND BLOOD PRESSURES DURING ACTIVITY AND DURING REST, WITH A NOTE ON CERTAIN ACUTE INFECTIONS AND ON THE DISTRESS RESULTING FROM EXERCISE<sup>1</sup>

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## INTRODUCTION

There are two methods of conducting an inquiry into the effects of physical exercise. On the one hand one may make a study of a very large number of subjects and average the results. The objection to this method is that it assumes that the numerous irrelevant variables, of which no account is taken, cancel one another so that the result indicates the action of a single common factor (exercise) and of its variations. On the other hand one may make an intensive study of a very few subjects. The objection here is that we are prone to assume that what is true of these few subjects is true of all potential subjects, whereas the cases studied may be exceptional ones.

To those who do not forget the limitations of these methods both are useful, suggestive and devoid of dangers. In the present instance the writer has chosen the second of the two, and has made an intensive study of a single subject, namely himself.

## THE SUBJECT

The subject's history in so far as it has any possible bearing on the present research is as follows: 1892-1895 intercollegiate athletics (Lacrosse); 1896-1905 in winter, excepting two months in mid-winter, vigorous exercise (one to two mile run several times a week); 1896-1907 in summer very strenuous mountain climbing sometimes involving feats of endurance. Since these dates systematic exercise has been

<sup>1</sup> A preliminary report of this work was made at the annual meeting of the American Physiological Society, December 1916 (5).

moderate and only for relatively short periods. Alcohol rarely and then in moderation, since 1907 not at all; no tobacco; coffee has been used occasionally. Between 1893 and 1911 acute dilatation occurred on four occasions. Recovery was always complete and prompt. Age 41 years in 1914 when these experiments were begun.

A partial physical examination of the subject made on April 27, 1916, gave the following data of possible relevancy to this research: Height 182.3 cm. weight 66.5 kilos (on June 7, 1916, 67.5 kilos); heart silhouette area 119.9 cm.<sup>2</sup> which is too small for age (43 years) and height, but normal for body weight according to Bardeen's tables. The estimated systolic out-put was 41 percent (Bardeen). A brief discussion and skiagraph of this heart has already been published (1).

#### METHOD OF MAKING OBSERVATIONS

The *blood pressures* were determined by means of the auscultatory method. Whenever the routine determinations were taken during rest (tables A, B and C) from five to twelve (usually about seven) separate observations were made of both systolic and diastolic pressures. This precaution eliminated variations due to respiration and possible Mayer's waves.<sup>2</sup> If the first systolic readings were markedly higher than those which followed, these first readings (with corresponding diastolic readings) were rejected on the ground that their exceptionally high level was probably the result of previous exertion so that they were not comparable with the subsequent readings. In regard to this matter a considerable amount of care has always to be exercised since the systolic pressure may be influenced by activities which appear quite inconsiderable, as going up or down stairs, going from room to room, or even fetching and arranging the necessary apparatus. The satisfactory readings were averaged and it is these averages which are used in the compilation of the accompanying tables (tables A, B and C). All the calculations of the averages were performed twice<sup>3</sup> independently to eliminate errors.

The counting of the *pulse rate* (one minute period) always followed immediately after the blood pressure determinations when the latter were made. When for any reason they were omitted, the pulse was counted until its rate became constant.

<sup>2</sup> The writer must again protest against the use of the term "Traube Herring wave" to designate vasomotor waves which are *not* synchronous with the respiratory movements (6), (7), (9).

<sup>3</sup> I have much pleasure in thanking my daughter Emily for performing the tedious task of making one of the two series of calculations.



While making these observations the subject remained comfortably seated. In no case was there demonstrable any psychical variation of either pulse or pressure. As a matter of fact there were usually no emotions accompanying the observations except occasionally an intense impatience and desire to have done with the readings. This state of mind appeared not to affect the readings at all.

The observations of pulse and blood pressure fall naturally into two categories: the daily routine observations and the special observations made in connection with the special physical tests and exercises.

The routine observations may be divided into four sets according to the time of day at which they were taken. These times were *a*, immediately after rising (about 6 a.m.); *b*, just before lunch (12 noon); *c*, late afternoon (4:30 to 6 p.m.); *d*, before retiring (9:30 to 11:30, usually 10 p.m.). These four sets are referred to in this paper as "morning," "noon," "afternoon" and "evening" respectively. These routine examinations show the changes in the resting pressure and pulse rate which were induced by systematic exercise.

In addition to these there was, as stated above, another category of observations, namely those which were made in connection with specific exercises, during and after riding the cycle ergometer and those made after the three-mile runs. In both cases the observations made before the exercises were part of the daily "routine" observations. These observations may be designated as "special" to distinguish them from the "routine" observations.

#### MODES OF EXERCISE

*Series I.* From December, 1913, to April 1, 1914, the subject took no exercise whatever. Soon after the latter date, however, he began to take exercise at first gently, later with some vigor. The routine observations are given in table A. The dates of occurrence of the exercise are seen in the accompanying calendar (1914). The exercise generally consisted in alternately walking and running for a distance of about three miles. As the strength of the subject improved he soon began to run the entire distance. It should be noted that owing to the age and prudence of the subject, the run was at a leisurely pace so that it required about thirty minutes for its accomplishment. There was no attempt made to shorten the time. The run ended in a short sprint which increased in vigor as the condition of the subject improved, but which did not appreciably shorten the time of the whole run. Both

before and after the period of systematic exercise (three-mile run) special tests were made by means of the bicycle ergometer. On these occasions the blood pressures and pulse rate were determined before, during<sup>4</sup> and after the ride. The pulse rate during the test was obtained in part by palpation, in part from a record of the carotid pulse obtained by means of tambours with air transmission. No observations were made during the run, but only before and after. Those of the former period were in reality a part of the daily routine examinations already referred to. The method of securing the observations after exercise was the following:

On returning from the run the subject at once dropped into an arm-chair and in about one-half minute was beginning to make blood pressure determinations. These were made in rapid succession for several minutes. Meanwhile an assistant (the subject's wife, to whose assiduity the writer is much indebted) adjusted the cuff of the Erlanger apparatus to the subject's ankle and in this manner obtained a graphic record of the pulse rate. The desirability of making a graphic record of the pulse rate depends upon the fact that after exercise the rate of the pulse falls from its maximum too rapidly to be estimated by means of the watch and palpating finger. One must therefore measure the duration of single beats or of small groups of beats.

These facts are gathered together in the accompanying calendar (1914).

#### *Calendar 1914*

All exercise ceased early in December.

*April 1.* First ride on cycle ergometer. Special observations were made during and after ride.

*April 2.* Daily routine observations of pressure and pulse rate begun.

*April 5 to April 20.* Series of runs and walks in preparation for three mile runs. Special observations made only after runs.

*April 22 to May 27.* A series of three-mile runs, 14 in 35 days. Special observations made only after runs.

*May 29 and June 8.* Second and third rides on the cycle ergometer respectively. Special observations made during and after ride.

*June 2.* Daily routine observation discontinued.

*Series II.* In 1915 the observations were begun on March 5 and ended May 5. From early in December to March 13, the subject took no exercise whatever. He then began a systematic daily performance of

<sup>4</sup> During the test the blood pressure observations were made by Professor Eyster, assisted by Miss Cantril. The latter embodied a presentation of a part of this work in her Master's thesis (2).

J. P. Müller's exercises in a moderated form (8). Later the exercises became more vigorous, but never reached in severity and tempo the standard set by Müller.

No special tests were made with the bicycle ergometer or in other ways, but routine observations were made of blood pressures and pulse rate from March 5 to April 19 (table B).

These facts are gathered together in the accompanying calendar (1915).

#### *Calendar 1915*

All exercise ceased early in December.

*March 5.* Daily routine observations of blood pressures and pulse rate begun.

*March 13 to April 13.* Modified Müller's exercises.

*April 19.* Daily routine observations discontinued.

*Series III.* In 1916 observations were begun on March 30 and continued until June 17 (table C). From December 1, 1915, to April 14, 1916, the subject had gone without exercise. On April 14 systematic exercises were begun. They consisted in riding upon the bicycle ergometer. On May 7 this exercise was discontinued. On June 6 and 7 two tests were made by means of the bicycle ergometer. Here, besides the daily routine observations of the pressures and pulse rate, the pressures (but not the pulse rate) were determined immediately after the rides upon the cycle ergometer in a manner similar to that described in the case of the three-mile runs undertaken in 1914. It should be noted in passing that the observations in 1916 were affected but not interrupted by two "colds," one at the beginning and one near the end of the period of observation. These facts are gathered together in the accompanying calendar (1916).

#### *Calendar 1916*

All exercise ceased early in December.

*March 30.* Daily routine observations begun.

*April 3.* First acute infection begins.

*April 14 to May 7.* Six rides on cycle ergometer followed by special determinations of blood pressures.

*May 9 to June 3.* Tennis.

*June 6 and June 7.* Two rides on cycle ergometer followed by special determination of blood pressures.

*June 8.* Second acute infection.

*June 17.* Daily routine observations discontinued.

## ABBREVIATIONS

Since it is highly important that the reader should always have in mind just what is implied in the reference to, let us say, the series of observations performed in 1914, namely, that in this year the form of exercise taken consisted in a three-mile run, the writer has resorted to the following way of designating the three series of experiments, namely: '14-run, '15-gym, '16'-cy-ten, where the suffixes run, gym (gymnastics), cy (cycle ergometer) and ten (tennis) signify the variety or varieties of exercises peculiar to the series mentioned.

## RESULTS I

*Effect of training on the resting pulse rate.* The effect of training upon the pulse of the subject while at "rest," that is to say, while doing only the ordinary work of the day and being therefore quite uninfluenced by any active physical exercise, was, with a single exception, to cause a decrease in the rate amounting to 3 to 9 beats per minute. (See table 1 and fig. 1.) The exception referred to is the rate on rising of series '15-gym where there is a slight increase in rate (one beat per minute). The rise is too small to lie beyond the limits of error but it is significant that in the morning observations of this series there was no decrease in rate. The exceptional nature of this result is probably due to the fact that in this series the exercise was exceptionally light (modified Müller's system).

TABLE 1  
*Effect of training on the resting pulse rate*

| SERIES           | NUM-<br>BER OF<br>OBSER-<br>VATIONS | TIME OF DAY | DATES (BEFORE)     | AVER-<br>AGE<br>PULSE<br>RATE |       | DATES (AFTER) |
|------------------|-------------------------------------|-------------|--------------------|-------------------------------|-------|---------------|
|                  |                                     |             |                    | Before                        | After |               |
| '14-run..... {   | 9                                   | Noon        | April 2-25         | 54                            | 51    | May 10-June 2 |
|                  | 3                                   | Afternoon   | April 2-4          | 61                            | 52    | May 31-June 2 |
| '16-cy-ten.... { | 10                                  | On rising   | March 30-April 10* | 58                            | 52    | May 29-June 7 |
|                  | 10                                  | Noon        | April 1-17†        | 58                            | 50    | May 29-June 7 |
| '15-gym..... {   | 6                                   | On rising   | March 6-12         | 55                            | 56    | April 12-17** |
|                  | 7                                   | Noon        | March 5-12         | 61                            | 55    | April 8-19    |



*Table 1.* The table shows that in the *first* column the series (date) and variety of exercise, given in order of the decreasing severity of the exercise; in the *second*, the number of observations from which the averages were obtained; in the *third*, the time of day at which the observations were made; *fourth*, the period (dates) during which observations were made prior to or early in the beginning of training; *fifth* and *sixth*, the average pulse rates corresponding to the dates given in columns four and seven respectively; *seventh*, the period (dates) during which the observations were made late in or subsequent to the period of training. \* Omitting April 3 and 4 on account of sickness. \*\* Omitting April 13 when exercise immediately preceded the routine observations. † Omit-

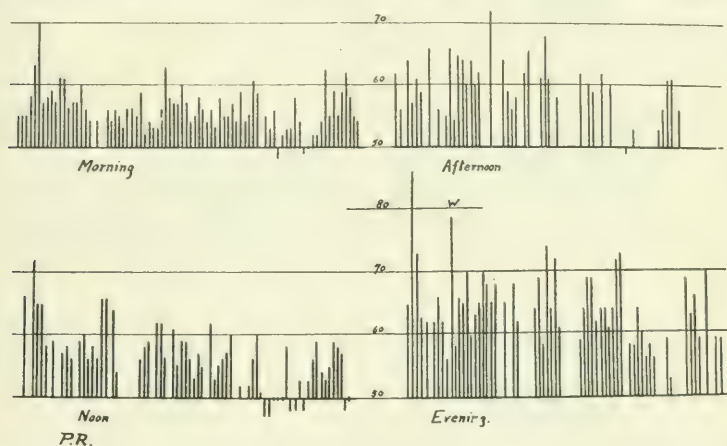


Fig. 1. Effect of training upon the resting pulse rate. The data presented in this figure are from series '16-cy-ten, table C. The lines in this figure represent pulse rates above or below 50 beats per minute. The lines are gathered into four groups corresponding to the time of day at which the observations were made. The observations in each group are arranged in order of time from left to right and wherever an observation is omitted a space has been left. Where the rate was just 50 per minute, a small stroke has been placed crossing the "50-line." Note the gradual decrease in the pulse rate with a slight increase at the end, the latter marking a corresponding decrease in the amount of physical exercise.

ting April 3, 4, and 5 on account of sickness. Evening observations which were influenced by previous periods of exercise, have not been used in making the averages. Note the decrease in the pulse rate after training.

*Effect of training on the resting blood pressures.* The effect of training on the resting blood pressures systolic and diastolic, is neither striking

nor constant at least not with exercises of the moderation employed in these experiments. Some changes have been noted of small amount and uncertain significance. For example, the behavior of the diastolic pressure in '14-run and '16-cy-ten is such that whether it falls or rises (the direction of the change depending upon the time of day at which the observations were made) there results an approach to 80 mm. (Table 2 and figs. 2 and 3). In this respect the noon observations of

TABLE 2  
*Effect of training on the resting blood pressures*

| SERIES          | NUMBER<br>OF OBSER-<br>VATIONS | TIME OF DAY | DATES (BEFORE) | AVERAGE BLOOD<br>PRESSURES |    |       |    | DATES (AFTER)      |
|-----------------|--------------------------------|-------------|----------------|----------------------------|----|-------|----|--------------------|
|                 |                                |             |                | Before                     |    | After |    |                    |
|                 |                                |             |                | S.                         | D. | D.    | S. |                    |
| '14-run.....    | 6                              | Noon        | April 2-13     | 121                        | 90 | 116   | 88 | May 23-<br>June 30 |
|                 | 3                              | Afternoon   | April 2-4      | 123                        | 97 | 120   | 90 | May 27-29          |
| '16-cy-ten..... | 10                             | On rising   | April 6-15     | 102                        | 76 | 106   | 77 | June 8-17          |
|                 | 10                             | Noon        | April 6-18     | 115                        | 85 | 115   | 82 | June 8-17          |
|                 | 5                              | Afternoon   | April 5-10     | 115                        | 84 | 117   | 82 | June 6-11          |
|                 | 7                              | Evening     | April 4-17     | 112                        | 78 | 116   | 78 | June 8-17          |
| '15-gym.....    | 6                              | On rising   | March 6-12     | 111                        | 85 | 110   | 80 | April 12-17        |
|                 | 10                             | Noon        | March 5-15     | 112                        | 84 | 120   | 91 | April 8-19         |

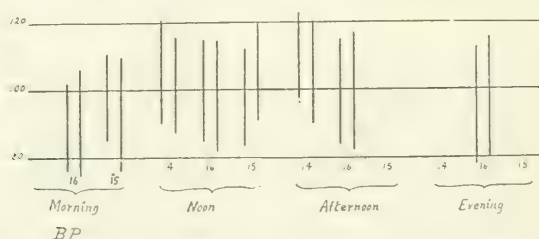


Fig. 2. Effect of training on the resting blood pressures. The numerals at the extreme left represent mm. Hg. The vertical lines are the pulse pressures, the upper end of each being at the systolic level, the lower at the diastolic level. The numerals placed below designate by dates the series of experiments to which the two lines immediately above each belong. Of each pair of vertical lines the one to the left represents the pressure before training; that to the right, the pressures after training. The series (date numerals) are arranged in order of decreasing severity of the exercise. Note the absence of any conspicuous or constant effect of training upon the systolic and diastolic pressures; at least when the exercise is of the moderation represented by these experiments. Possibly the diastolic pressure tends to approach 80 mm.

'15-gym differ from the other two series but whether this was due to the difference in kind or in severity of the exercise or to some other cause, is purely conjectural. It is also to be noted that the change in the relation of the systolic to the diastolic pressure in all three series was usually such as to increase the pulse pressure (*vide infra*).

*Table 2.* The table shows in the *first* column the series (date) and the variety of exercise, given in the order of the decreasing severity of the exercise; in the *second*, the number of observations from which the averages were obtained; *third*, the time of day at which the observations were made; *fourth*, the period (dates) during which observations were made prior to or early in the beginning of the training; *fifth and sixth*, the average systolic and diastolic pressures corresponding to the dates in columns four and seven respectively; *seventh*, the period (dates) during which observations were made late in or subsequent to the period

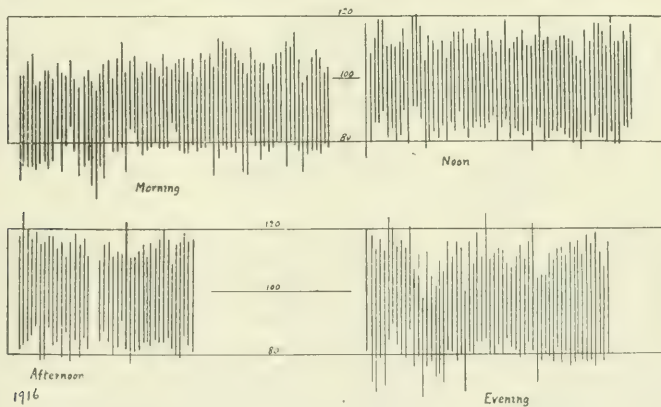


Fig. 3. Development of the effect of training upon the blood pressures. In as much as the observations are more complete for '16-cy-ten than for either of the other series, it has seemed justifiable to devote to their presentation a separate figure. This figure is based upon the entire number of observations comprised in the series in question (table C). In this figure the vertical lines are the pulse pressure, the upper end of each being at the systolic level, the lower end at the diastolic level. The horizontal lines of reference represent 120 mm. Hg. (upper line) and 80 mm. Hg. (lower line) respectively. The vertical lines are divided into four groups corresponding to the observations made at different times of the day. The changes in the pressures are appreciated best when the observer looks at the chart from the side and foreshortens it by holding it at an obtuse angle to the line of vision. *Note the gradual developments of the changes in the blood pressures, of which figure 2 gives only the final outcome.* One may also obtain from this chart some idea of the character of the *diurnal variations in the blood pressures*, to which special reference will be made later.

of training. The evening observations which were influenced by previous periods of exercise, have not been used in making the averages.

*Effect of training on the resting pulse pressures.* The effect of training upon the pulse pressure during rest is almost always in the direction of an increase. Sometimes this increase is small (3 mm.), at others larger (6 mm.), but in only one instance was there a decrease (3 mm.) namely in '14-run, noon. (Table 3 and also fig. 4.) The writer has been unable to assign a plausible reason for the exception mentioned above. Numerically it was due, at least in part, to a period of high systolic pressure during which the series '14-run was begun to a period of high diastolic pressure during the last few days of the series.

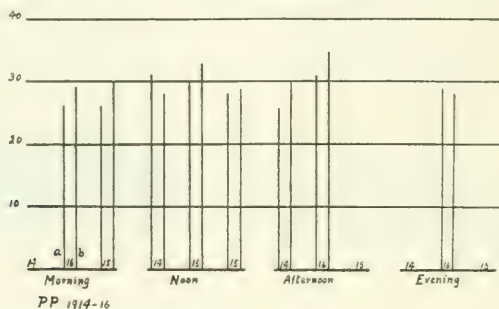


Fig. 4. Effect of training on the resting pulse pressure. The values used in this figure are the same as those presented in table 3. The numerals at the extreme left and right represent mm. Hg. The vertical lines are the pulse pressures. The numerals placed below indicate the series (dates) to which the two lines immediately above belong. Of each pair of vertical lines, the one to the left represents the pulse pressures before training and that to the right the pulse pressures after training. The date numerals are arranged in order of the severity of the exercise from left to right. Note that in almost every case there is an increase in the pulse pressure as the result of training, but that the increase is often quite small.

*Effect of training on the product  $P. R. \times P. P.$  during rest.* The product of the pulse rate times the pulse pressure is of interest since it is possibly (3), (4) an index of "minute volume" (output of the heart per minute). It has been seen above that as a result of training the pulse rate falls while the pulse pressure rises in value. The product would consequently tend to remain unchanged and would be entirely unchanged if the variations in rate and pulse pressure were proportional as well as being in the opposite direction. This is, however, not the case. The product may be found to have risen or fallen so that the compensation (if we may speak of such) is not exact but sometimes falls short and sometimes



TABLE 3

*Effect of training on the pulse pressure and on the product P. R.  $\times$  P. P. during rest*

| SERIES          | TIME OF DAY | PULSE RATE $\times$ PULSE PRESSURE |                    |       |                  |
|-----------------|-------------|------------------------------------|--------------------|-------|------------------|
|                 |             | Before                             |                    | After |                  |
|                 |             | P. R.                              | P. P.              | P. R. | P. P.            |
| '14-run.....    | Noon        | 55                                 | $\times 31 = 1705$ | 1512  | $= 54 \times 28$ |
|                 | Afternoon   | 61                                 | $\times 26 = 1580$ | 1740  | $= 58 \times 30$ |
| '16-cy-ten..... | On rising   | 58                                 | $\times 26 = 1508$ | 1653  | $= 57 \times 29$ |
|                 | Noon        | 58                                 | $\times 30 = 1740$ | 1815  | $= 55 \times 33$ |
|                 | Afternoon   | 60                                 | $\times 31 = 1860$ | 1991  | $= 57 \times 35$ |
|                 | Evening     | 62*                                | $\times 34 = 2108$ | 2356  | $= 62 \times 38$ |
| '15-gym.....    | On rising   | 55                                 | $\times 26 = 1400$ | 1680  | $= 56 \times 30$ |
|                 | Noon        | 60                                 | $\times 28 = 1680$ | 1595  | $= 55 \times 29$ |

is excessive. An increase in the product was of more frequent occurrence than a decrease. These differences are very slight and the preponderance of the increase over the decrease may not be significant. (Table 3, and fig. 5.)

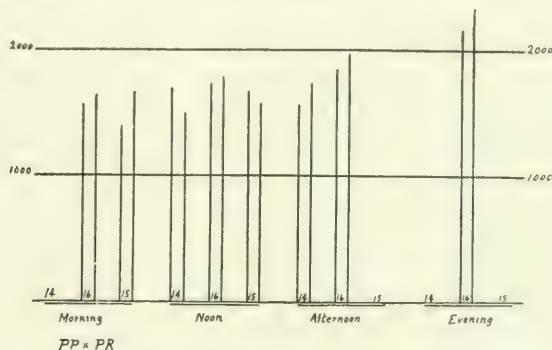


Fig. 5. Effect of training on the product of the pulse rate and pulse pressure P. R.  $\times$  P. P. during rest. This product is of interest from the fact that it may represent the cardiac output per minute (minute volume). The data from which this figure was constructed are those presented in table 3. The numerals at the extreme left and right represent mm. Hg. The vertical lines represent the product P. R.  $\times$  P. P. The numerals placed below indicate the series (dates) of experiments to which the two lines immediately above belong. Of each pair of vertical lines the one to the left represents the product before training and that to the right the product after training. The series (date numerals) are arranged in order of the decreasing severity of the exercise from left to right. Note that in almost every case there is an increase in the product as the result of training although this increase is often quite small.

There is in the product under consideration a diurnal variation of the nature of an increase throughout the day. This variation is apparently not affected by training.

*Table 3.* The table shows in the *first* column the series (date) and the variety of exercise, given in the order of the severity of the exercise; *second*, the time of day at which the observations were made; *third*, the pulse rates corresponding to the pulse pressures in the next column; *fourth*, the pulse pressure before or early in the period of training, derived in great measure from the data given in table 2; *fifth*, the product of columns three and four; *sixth*, the product of columns seven and eight, placed next to column five for convenience of comparison; *seventh*, pulse rates corresponding to pulse pressure in the last column; *eighth*, the pulse pressure after or late in the period of training, derived in great measure from the data given in table 2. \*From April 2-11. *Note that the pulse pressure and the values of  $P. R. \times P. P.$  are usually greater "after" than "before."*

*Table 4.* The data are from '16-cy-ten. This table shows in the *first* column the dates upon which the observations were made. On these dates there was no afternoon exercise or other unusual event to influence the evening values; the *second* to *fifth* columns are pulse rates and require no explanation. The figures are divided into two sets (April 6 to May 2 and May 6 to 31). The first set occurs in the earlier part of the period of training, the second set in the latter part. Below these columns are placed the averages. *Note that the pulse rate shows no striking change which might be attributed to training in the character of the diurnal variations but that the extent of the variations is slightly increased.*

*Effect of training on the diurnal variations of the resting pulse rate.* The effect of training upon the resting pulse rate is not of the same magnitude for all times of the day (table 4). The most pronounced slowing is that of the pulse rates for noon and late afternoon, namely, five and four beats respectively as compared with three beats per minute in the morning and two in the evening. An unequally distributed change of this kind would naturally alter the *form* of the diurnal pulse curve. This alteration is, however, so small that it may lie entirely within the limits of error.

The curve is altered also in its *extent*, for if the comparison be made between the amount of the average daily variation before and after training, the greater variation is found to occur after training. The increase in variation is, however, very small, amounting to only 1.3 beats and may well lie within the limits of error or if real be too small to be significant.

TABLE 4

*Effect of training on the diurnal variations of the pulse rate during rest*

| DATE                  | MORNING | NOON | AFTERNOON | EVENING |
|-----------------------|---------|------|-----------|---------|
| April 6.....          | 58      | 58   | 61        | 62      |
| April 11.....         | 56      | 58   | 56        | 56      |
| April 17.....         | 50      | 58   | 64        | 60      |
| April 19.....         | 54      | 66   | 64        | 65      |
| April 20.....         | 56      | 66   | 60        | 70      |
| April 28.....         | 54      | 56   | 59        | 62      |
| May 2.....            | 63      | 62   | 62        | 64      |
| Average (before)..... | 58      | 61   | 63        | 63      |
| May 6.....            | 60      | 61   | 61        | 64      |
| May 8.....            | 54      | 59   | 61        | 61      |
| May 17.....           | 55      | 55   | 62        | 62      |
| May 19.....           | 54      | 57   | 60        | 64      |
| May 20.....           | 59      | 60   | 59        | 61      |
| May 29.....           | 48      | 47   | 49        | 56      |
| May 31.....           | 53      | 50   | 53        | 56      |
| Average (after).....  | 55      | 56   | 57        | 61      |

*Effect of training on the diurnal variations of the blood pressures during rest.* The diurnal variations of blood pressures in one series of observations ('16-cy-ten) are also shown in figure 3. From this series there have been selected all the complete diurnal cycles which have not been interfered with in respect to the evening observations by a period of exercise preceding the latter or by any other disturbing influence. When this was done the number of such cycles was found to be fourteen.

The variations may be regarded from two points of view. First, one may consider the separate daily curves to note any variation in the form of these curves; and second, one may compare all the absolute values of the pressures at different periods of the day. (For example, we may compare all the noon pressures).

If we examine the daily curves (fig. 6) it is found that the systolic pressures show seven different rhythms (table 5) of which two predominate, namely, *a*, a rise to a maximum at noon followed by a fall in the afternoon and evening; and *b*, the noon maximum is sustained until afternoon and the fall is not observed until evening.

*Table 5.* The data from which this table is derived are those which have formed the basis of figure 6. They represent observations made on fourteen days of the series '16-cy-ten. The table shows in the *first* column the number of instances in which the rhythm, which immediately follows, has occurred; in the *second*, the character of the change which took place between the morning and the noon observations; the *third* column, between the noon and the afternoon observations; the *fourth*, between the afternoon and the evening observations. *Note that the usual systolic rhythms are "up-no change-up" and "up-up-down," while the usual diastolic rhythms are "up-down-down" and "up-up-down."*

On the other hand the changes in the diastolic pressure are more constant. Here only three rhythms occur (table 5) one of which appears but once. The remaining two are, *a*, a rise to noon and then a fall; *b*, a rise to afternoon and then a fall.

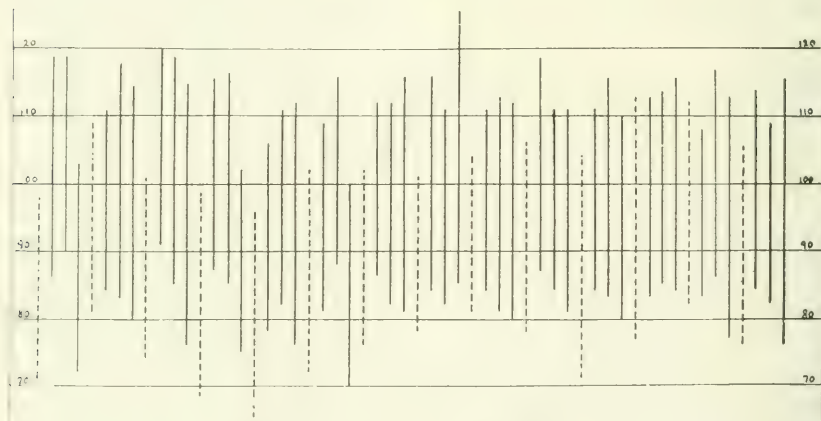


Fig. 6. The daily variations in systolic, diastolic and pulse pressure during training. The observations from which this figure has been constructed were made on fourteen days of the series '16-cy-ten. The numerals at the extreme left and right represent mm. Hg. Vertical bars are pulse pressures, upper end of each being at systolic level, lower at the diastolic. Dotted bars represent morning pressures. *Note increase in diurnal variations of systolic pressure, decrease in diurnal variations of diastolic pressure, and that the character of the daily rhythms is unchanged.*

The pulse pressure corresponding to the systolic and diastolic pressures just mentioned show daily rhythms which are remarkably variable. In these fourteen days there are twelve different rhythms of which two are repeated (making the total of fourteen cycles).

There seems to be no evidence in these qualitative data that training produces any effect upon the inter-relations of the four points on the



diurnal curves of systolic, diastolic or pulse pressure. In other words, we do not find that one form of diurnal curve predominates before training while another predominates afterwards.

The effect of training upon the absolute values of the blood pressures observed at different times of the day has already been referred to. By way of recapitulation it may be said that (omitting '15-gym) the morning pressures are raised, the noon lowered while the afternoon and evening pulse pressures are increased. Moreover there is a gradual reduction of the amount of diurnal variation of the blood pressures (as shown in fig. 6).

The amount of this reduction of the diurnal variations may be expressed as follows. If we observe the values of the systolic pressure

TABLE 5  
*Diurnal rhythms in systolic and diastolic pressure*

|                         | NUMBER OF<br>INSTANCES | MORNING TO<br>NOON | NOON TO<br>AFTERNOON | AFTERNOON TO<br>EVENING |
|-------------------------|------------------------|--------------------|----------------------|-------------------------|
| Systolic pressure.....  | 4                      | Rise               | No change            | Fall                    |
|                         | 4                      | Rise               | Rise                 | Fall                    |
|                         | 1                      | Rise               | Fall                 | Rise                    |
|                         | 1                      | Rise               | Fall                 | No change               |
|                         | 1                      | Rise               | Rise                 | Rise                    |
|                         | 1                      | Rise               | No change            | Rise                    |
|                         | 1                      | Fall               | Rise                 | Fall                    |
| Diastolic pressure..... | 8                      | Rise               | Fall                 | Fall                    |
|                         | 5                      | Rise               | Rise                 | Fall                    |
|                         | 1                      | Rise               | Fall                 | Rise                    |

for one day, and subtract the minimum systolic pressure from the maximum systolic pressure we get a figure indicative of the difference between the extremes of systolic variation on this day. If the values obtained for several days selected from the period preceding or early in training be compared with the values similarly selected from a period later in or subsequent to the training, then the latter are found to be less than the former by 20 per cent. If the values of the diastolic pressure be treated in the same manner, the variations after training are found to have decreased by 50 per cent. Finally if the difference between the maximum and minimum pulse pressure be considered, one finds a decrease of 10 per cent only, which the writer does not think is large enough to be safely beyond the limits of error. Consequently, although there is as has been shown an increase in the pulse pressure

after training, yet the diurnal fluctuations in the size of the pulse pressure have not been positively shown to vary. The product of P.R.  $\times$  P.P. shows a diurnal variation of the nature of an increase throughout the day. The character of this variation seems not to be affected by training.

## RESULTS II

*Effect of training and practice on the reaction to exercise of blood pressures and pulse rate.* The reaction to exercise depends upon two factors. If a subject be tested on a bicycle ergometer and then runs several miles every few days for several weeks, and if he be finally tested again with the ergometer, the change in his reaction is due to an improvement in his general physical condition which is the result of this systematic exercise. This state or condition we have called "training." If on the other hand the reaction of the subject to the first run be compared with the reaction of the subject to the last run, then the change is attributable to two factors: first, to what has been called above "training," and second, to "practice." The latter is in all probability a condition of neuromuscular adaptation to a certain special sort of exercise, in the case cited, running. The reaction to exercise is modified by these two factors. If the subject is in training or has practiced he reacts in one way; if not, he reacts differently. It is these differences which constitute the topic of the present section.

That practice and training are quite different states of efficiency is shown by such observations as the following: A young woman who was an expert swimmer and possessed great physical strength, was seized with an acute dilatation of the heart on her first attempt at mountain climbing, the tax not being a severe one. By beginning again with great caution after a few days of rest, and progressively increasing the severity of the climbing, she became at the end of a few weeks as efficient in this form of exertion as in those to which she had already been inured, namely, rowing and swimming. Here the subject though trained was not practiced. And the result when practice had been added to training was quite different from the effect when the condition was one of training only. It is not possible to separate with entire satisfaction the effects of practice from those of training and practice combined, but it is readily possible to separate the effect of training from this combination as has been intimated.

It is exceedingly important to remember that throughout the present study of the reaction to exercise before and after training, *the amount of*

*exercise is not a constant factor.* In the beginning of the experiments it was thought desirable to keep the amount of work done as nearly constant as possible. That would have made the results obtained show the effect of practice and training on the circulatory reaction to a fixed amount of work. But in the enthusiasm and delight of physical activity, it was found unbearably irksome to restrain the trained body. In every cycle ride the subject when once underway proceeded forthwith to pedal joyously. In the case of the three-mile run the natural beauty of the environment proved a sufficient distraction to permit an almost uniform rate of progression, but the approaching of home called forth an inevitable sprint which increased in vigor with training and practice. What these experiments show is the effect of training and practice upon the reaction of the pulse rate and blood pressures of a subject who (within the limits imposed by prudence) performs as vigorously as possible on every cycle ride and three-mile finish.

With the recently improved facilities of this laboratory it is practically certain that studies will soon be made of the reaction to a standardized task, but it cannot be too frequently reiterated that the present article is *not* such a study.

It was found that the same qualitative results were obtained whether the observations were made during the latter part of the period of exercise or immediately after exercise. But quantitatively the changes noted after exercise were, as might be expected, just a little less in extent and the condition continued to approach normal with the lapse of time.

In some of the instances described in this section the observations were made before, during and after the test exercises (cycle ergometer), while in others they were made only before and after exercise (cycle ergometer and three-mile run).

The reaction of the blood pressures and pulse rate to exercise is well known. It consists in a rise in the systolic and pulse pressures and to a much less extent of the diastolic pressure. The pulse rate is also markedly increased.

*Effect of training only.* The effect produced by training on the circulatory reaction (fig. 7) is as follows: *a*, the systolic pressure rises more rapidly and much higher than is the case in the untrained individual; *b*, the diastolic pressure often returns to normal before the cessation of the exercise; *c*, the pulse pressure is enormously increased. Before training the rise in pulse pressure was from 38 (normal) to 62, after training from 37 to 114. *d*, The product P.R.  $\times$  P.P. is also greatly increased (before training the rise was from 2660 (normal) to 6820, after

training from 2220 to 12540). *e*, The change in the pulse rate is but little affected; *f*, moreover with less effort more mechanical work is done.

*Effect of practice only.* No systematic attempt was made to dissociate training from practice so that a separate study of the latter might be made. There was, however, an incidental observation which is worth recording. In the series '16-cy-ten, it was found that the blood pressure reaction after the second ride was remarkably different from that following the first ride. This change in the reaction was of the nature of an increase in the systolic pressure and pulse pressure and subjectively of a decrease in the discomfort.

It seems more improbable that a single ride should have put the subject into a condition of training than that he should have experienced

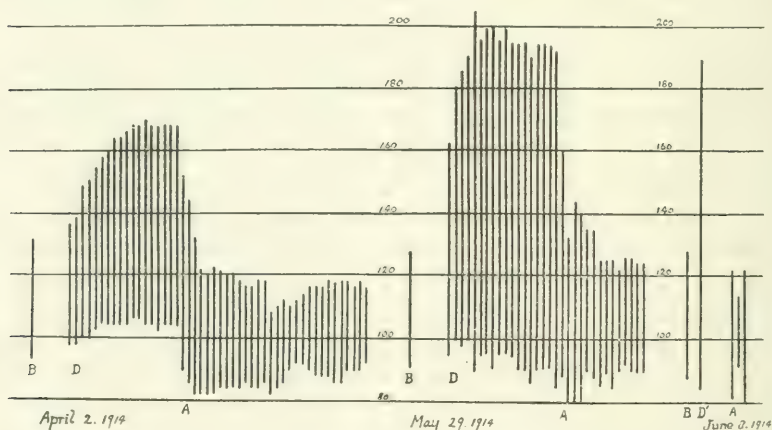


Fig. 7. Effect of training on the reaction of the pulse rate and blood pressures to cycle riding. The numerals in vertical columns represent mm. Hg. The vertical lines are the pulse pressures, the upper end of each being at the systolic level, the lower at the diastolic level. The letters *B*, *D* and *A* stand for "before," "during" and "after" respectively. April 1: immediately after *B*, the cycle ride began and observations were made at frequent intervals both during the ride, *D* and afterward, *A*. Here  $\text{kilos} \times \text{revolutions} = 7 \times 3276 = 23,849$  and  $\text{P. R.} \times \text{P. P.} = 110 \times 60 = 6820$ , during exercise. May 29: as before, but *S* is fifty-five minutes later than the end of *A*. Here  $\text{kilos} \times \text{revolutions} = 7 \times 4423 = 30,961$  and  $\text{P. R.} \times \text{P. P.} = 110 \times 114 = 12,540$ . June 8: *B* is as before but of the pressures recorded during exercise a single pair (that giving the maximum pulse pressure) is here drawn, *D*, and only the last three pairs of observations from the period after exercise are given, *A*. Note that after training there is an increase in the reaction to exercise in respect to the systolic and pulse pressures but a decrease in the diastolic pressure.



the beneficial effects of a little practice. But however this may be, the fact remains that a single ride produced a pronounced effect. (Table 6 and fig. 8).

TABLE 6

*Effect of practice and of training and practice on the reaction of the blood pressures to exercise (cycle riding)*

| DATE          | KILOS | REVOLU-<br>TION | K. X R. | MINUTES | BEFORE EXERCISE |    |      |      |                   | AFTER EXERCISE |    |      |                            |
|---------------|-------|-----------------|---------|---------|-----------------|----|------|------|-------------------|----------------|----|------|----------------------------|
|               |       |                 |         |         | S.              | D. | P.P. | P.R. | P.R.<br>X<br>P.P. | S.             | D. | P.P. | P.R.<br>X<br>P.P.          |
| April 14..... | 6     | 3900            | 23,400  | 25      | 114             | 78 | 36   | 66   | 2376              | 120            | 74 | 46   | (5060)<br>(5520)<br>(5980) |
| April 16..... | 6     | 5399            | 32,394  | 30      | 116             | 77 | 39   | 65   | 2535              | 140            | 80 | 60   | (6600)<br>(7200)<br>(7800) |
| April 29..... | 8     | 6113            | 48,904  | 30      | 111             | 84 | 27   | 56   | 1512              | 146            | 76 | 70   | (7700)<br>(8400)<br>(9100) |
| May 3.....    | 8     | 5790            | 46,320  | 30      | 112             | 90 | 32   | 66   | 2112              | 140            | 76 | 64   | (7040)<br>(7680)<br>(8320) |

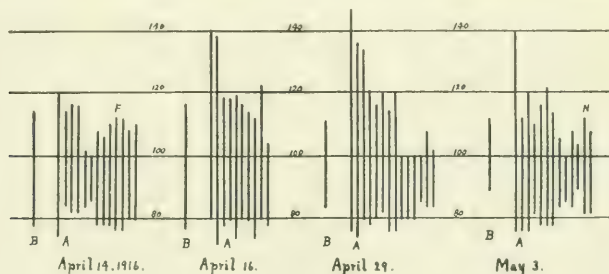


Fig. 8. Effect of practice and training on the reaction of the blood pressures to cycle riding. The data corresponding to this figure are shown in table 6. They are from the series '16-cy-ten'. The numerals placed in the vertical columns represent mm. Hg. The vertical lines are the pulse pressures, the upper end of each being at the systolic level, the lower at the diastolic level. The figure shows observations taken before B and immediately after A, riding the bicycle ergometer. At F, there was a transitory feeling of faintness; at N, an equally transitory feeling of nausea. The duration of the rides were in three cases thirty minutes, in one case (April 14) twenty-five minutes. Kilos X revolutions = (April 14) 23,400; (April 16) = 32,394; (April 29) = 48,904; (May 3) = 46,320. Note that after training the heart is capable of a much greater reaction without untoward symptoms.

*Table 6.* The data are from the series '16-cy-ten and the table corresponds to figure 8. In the *first* column are given the dates of the experiments; in the *second*, the weight of the brake, in kilos; *third*, the number of revolutions of the cycle ergometer; *fourth*, the product of the weight times the revolutions; *fifth*, the duration of the ride; *sixth* to *tenth* inclusive, blood pressures, pulse rate and their derivatives, taken before exercise; *eleventh* to *fourteenth* inclusive, the blood pressures, pulse rate and their derivatives observed after exercise with the exception to be mentioned immediately, namely, that as the P.R. was not observed, the numerals in parentheses were calculated after assuming a rate of 110, 120 or 130 beats per minute, and are presented as suggestive merely. Note that the practice and training exaggerate the increase in systolic pressure and pulse pressure and assuming that the difference in increase of the pulse rate was not more than twenty beats (cf. table 9) that the product  $P.R. \times P.P.$  is also increased.

TABLE 7

*Effect of training and practice on the reaction of the blood pressures and pulse rate to exercise (three-mile run)*

| DATE          | BEFORE EXERCISE |    |      |      |                    | AFTER EXERCISE |    |      |      |                    |
|---------------|-----------------|----|------|------|--------------------|----------------|----|------|------|--------------------|
|               | S.              | D. | P.P. | P.R. | P.R. $\times$ P.P. | S.             | D. | P.P. | P.R. | P.R. $\times$ P.P. |
| April 17..... | 128             | 90 | 38   | 58   | 2204               | 138            | 90 | 48   | 100  | 4800               |
| May 8.....    | 111             | 78 | 33   | 55   | 2815               | 164            | 80 | 84   | 96   | 8064               |
| May 14.....   | 110             | 83 | 27   | 65   | 1701               | 168            | 76 | 92   | 108  | 9936               |

*Combined effect of training and practice.* These effects were studied in two series of experiments. In the *first* ('14-run) observations were made of both pulse rate and blood pressures while in the second ('16-cy) the blood pressure only was determined. In both, the observations were made before and again immediately after exercise. In respect to the blood pressures, the results were essentially the same in both series and were in accord with those obtained by training alone. In the first series ('14-run) *a*, there was an increase in the rise of the systolic pressure; *b*, the change in diastolic pressure was not markedly affected; *c*, the pulse pressure was enormously increased; *d*, the product  $P.R. \times P.P.$  was also greatly increased. Also the mechanical work done was greater with apparently less exertion. *e*, The pulse rate was decreased (cf. table 9). Unfortunately the negative phases cannot be compared in this series because the late observations were not made at the same interval of time after the cycle rides. (Table 7, fig. 9).

*Table 7.* The data are from the series '14-run and the table corresponds to figure 9. The table requires no further explanation. *Note that the training and practice exaggerate the increase in systolic pressure, pulse pressure and the product  $P.R. \times P.P.$  while the change in the pulse rate is unaffected.*

In the *second* series ('16-cy-ten) the first exercise of the season was a cycle ride taking place on April 14. (Table 8, fig. 10). On April 29 the ninth cycle ride took place. On June 6 and 7 came the tenth and eleventh rides respectively. Between the first and ninth rides cycle

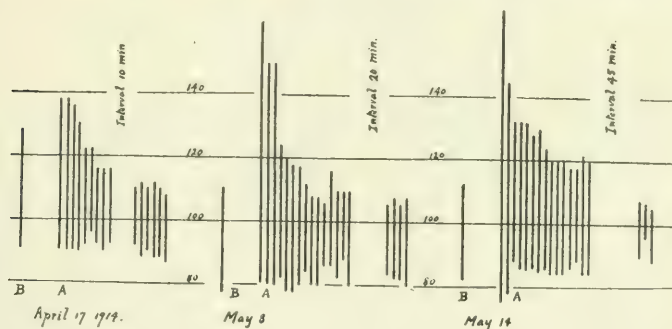


Fig. 9. Effect of training and practice on the reaction of the blood pressure to the three-mile run. The numerical data corresponding to this figure are shown in table 7. They are from series '14-run. The numerals in vertical columns represent mm. Hg. The vertical lines are the pulse pressures, the upper end of each being at the systolic level while the lower end is at the diastolic level. B indicates observations made immediately before and A, immediately after exercise. Here  $P.R. \times P.P.$  (April 17) = 4800; (May 8) = 8064; (May 14) = 9936. The observations made after the exercise are in each case interrupted for a few minutes as indicated in the figure. *Note that after training there is an increase in the reaction to exercise in respect to the systolic and pulse pressures.*

riding was the only form of exercise. Between the ninth and tenth rides there intervened several weeks of tennis playing. Consequently in the first period the cycle ergometer tested the effect of both training and practice while in the second period it tested only that of training. The second period, however, was not particularly significant because the subject had just passed through a course of exercise which had already removed most of the effects of the prolonged inactivity which had preceded the series. On comparing the effect of the first cycle ride with that of the ninth, one finds the same results as in the first series with respect to the blood pressure. Moreover in comparing the effect

of the ninth ride with that of the tenth and eleventh, one finds that the changes in the reaction to exercise are no greater after nine rides and several weeks of tennis than after nine rides alone. In the former case the negative phase is not quite so conspicuous as in the latter which suggests that the result of the further training may have been to permit an increase of this phase. This conclusion is nevertheless insecurely based since the negative phase, properly so called, would not be expected to occur until some time subsequent to the last observations made in each of the riding tests in question.

TABLE 8

*Effect of practice and training on the reaction of the blood pressure to exercise (cycle riding)*

| DATE          | KILOS | REVOLU-<br>TION | K. X R. | MINUTES | BEFORE EXERCISE |    |      |      |                   | AFTER EXERCISE |    |      |      |                            |
|---------------|-------|-----------------|---------|---------|-----------------|----|------|------|-------------------|----------------|----|------|------|----------------------------|
|               |       |                 |         |         | S.              | D. | P.P. | P.R. | P.R.<br>X<br>P.P. | S.             | D. | P.P. | P.R. | P.R.<br>X<br>P.P.          |
| April 14..... | 6     | 3900            | 23,400  | 25      | 114             | 78 | 36   | 66   | 2376              | 120            | 74 | 46   |      | (5060)<br>(5520)<br>(5980) |
| April 29..... | 8     | 6113            | 48,904  | 30      | 111             | 84 | 27   | 56   | 1215              | 146            | 76 | 70   |      | (7700)<br>(8400)<br>(9100) |
| June 2.....   | —     | —               | —       | —       | 117             | 80 | 37   | 53   | 1961              | 144            | 70 | 74   |      | (8140)<br>(8880)<br>(9620) |
| June 7.....   | 8     | 7007            | 56,056  | 30      | 115             | 78 | 37   | 56   | 2072              | 144            | 80 | 64   |      | (7040)<br>(7680)<br>(8320) |

*Table 8.* The data from '16-cy-ten and the table corresponds to figure 10. In the *first* column are given the dates of the experiments; in the *second*, the weight of the break; *third*, the number of revolutions of the cycle-ergometer; *fourth*, the product of the weight times the revolutions; *fifth*, the duration of the ride; *sixth* to *tenth* inclusive, blood pressures, pulse rate and their derivatives, taken before the exercise; *eleventh* to *sixteenth* inclusive, the blood pressures, pulse rate and their derivatives observed after the exercise, the exception to be mentioned immediately, namely, since the P.R. was not observed, the numerals in parentheses were calculated on the assumption of a rate of 110, 120 or 130 beats per minute, and are presented as suggestive merely. *Note that the practice and training exaggerate the increase in systolic pressure and pulse pressure, and assuming that the increase of the pulse rate is in-*



creased not more than twenty beats (see table 9) that the product  $P.R. \times P.P.$  is also increased.

From the *subjective side* the effect of training alone and of training and practice combined is to enable the heart to work more vigorously without untoward symptoms. Although before training or practice the pulse pressure (systolic output?) is relatively small during (and just after) exercise, the distress is considerable; later, after training or practice, although the pulse pressure is very much greater, the individual suffers no subjective embarrassment whatever.

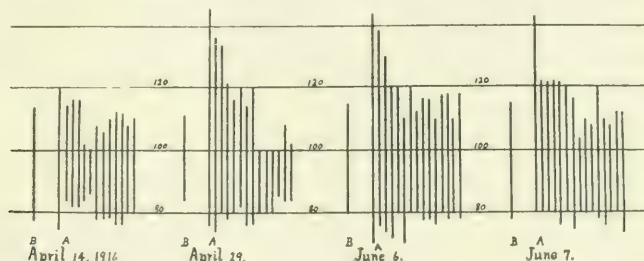


Fig. 10. Effect of practice and of training and practice (?) on the reaction of the blood pressure to cycle riding. The data corresponding to this figure are shown in table 8. They are from series '16-cy-ten. The numerals placed in the vertical columns represent mm. Hg. The vertical lines are the pulse pressures, the upper end of each being at the systolic level, the lower at the diastolic level. The observations were made before, *B*, and immediately after, *A*, riding the bicycle ergometer. Here kilos  $\times$  revolutions (April 14) = 23,400; (April 29) = 48,904; (June 7) = 56,056. The duration of the exercise was twenty-five minutes in one case (April 14), thirty minutes in the other two. Note that after training there is an increase in the reaction to exercise in respect to the systolic and pulse pressures.

*Effect of training and practice on the pulse rate after exercise and on its return to normal.* A few observations have been made with reference to the effect of practice and training *a*, upon the increase of the pulse rate due to exercise, and *b*, upon the rapidity of the return of the pulse rate to normal after exercise has ceased.

*a.* If the relevant data from series '14-run be divided into two parts, the first of which represents the results obtained early in the period of training and practice, and the second the data obtained later in this period, it is possible to make certain comparisons (table 9). If one compares the maximum rate after exercise during the first half period with the corresponding rate during the second half period, one finds that the former exceeds the latter (150 beats per minute as compared with 126). Again if one compares the average rate during the first half

period with the average rate during the second half period, one finds that once more the former exceeds the latter (119 and 127 respectively). It is quite probable that these differences would have been greater but for certain counteracting influences, namely *a*, that the final sprint was more vigorous as the condition of the runner improved, and *b*, that the pulse record was probably begun a trifle earlier (that is, with less loss of time) as the assistant became more deft in adjusting and inflating the cuff upon the ankle. It is possible that the fact that during the last two days (May 26 and 27) the weather was unusually hot may also have tended to raise the pulse. The resting pulse of this subject is faster in warm weather than in cool, and it may be that the pulse in reacting to exercise rises higher during warm than during cold weather, but this has not been ascertained.

*Table 9.* The data are from series '14-run and represent the pulse after a three-mile run. During the two hours referred to in column eight, the subject bathed and dined. The values given (pulse rates per minute) were calculated from the rates for 5 to 10 seconds, consequently all errors are highly magnified. This is why columns two and three, and four and five have been averaged together (average II). The values used in making the averages are enclosed in parenthesis. The value 132\* is so high that it arouses distrust as to its accuracy, it is therefore omitted from one average and included in the other (\*). *Note that the averages are lower "immediately after" running in the trained and practiced subject, but that at the end of two minutes this difference has considerably decreased.*

*b.* After exercise the pulse rate falls at first rapidly and then more slowly. Even at the end of five hours it was still above normal as can be seen from the following values, namely, 65.5 (62 if we omit the warm days May 26 and 27). In series '14-run, the pulse rate at the time of the retiring was 65.5 (62 if we omit May 26 and 27, when the weather was unusually warm) on days on which the subject exercised, while on the resting days, the corresponding figure was only 56. This tardiness in the return of the pulse to normal is seen again in series '16-cy-ten. Here the average pulse rate on retiring was 68 on days of cycle riding and on days of resting 62. (Table C).

For comparing the readiness with which the pulse rate returned to normal during the first part of the period of training with the corresponding values for the second part, one turns again to series '14-run. Here observations made *two minutes after cessation* of exercise were divided into two sets corresponding to the first part of the period of

training, and to the second part respectively. Such a comparison (table 9) shows that the difference between the averages of the rates during these two half-periods has decreased below what it was immediately after exercise. In other words, the higher pulse rate has fallen more rapidly than the lower although neither had of course as yet reached the normal level.

TABLE 9

*Effect of training and practice upon the return of the pulse rates to normal after exercise*

| DATE            | IMMEDIATELY<br>AFTER<br>RUN | 10<br>SECONDS | 1 MINUTE    | 2<br>MINUTES | 3<br>MINUTES               | 5<br>MINUTES | ABOUT 2<br>HOURS | ABOUT 5<br>HOURS |
|-----------------|-----------------------------|---------------|-------------|--------------|----------------------------|--------------|------------------|------------------|
| April 17.....   | 100                         |               |             |              | 80                         |              |                  |                  |
| April 18.....   | (102)                       | (96)          | (90)        | (80)         |                            |              |                  |                  |
| April 27.....   | (150)                       | (150)         | (114)       | (92)         | 88                         |              |                  |                  |
| April 30.....   | (132)                       | (132)         | (108)       | (94)         |                            |              |                  |                  |
| May 1.....      | (150)                       | (114)         | (132)*      | (92)         |                            |              |                  |                  |
| May 2.....      | (120)                       | (102)         | (108)       | (96)         |                            |              | 71               | 56               |
| May 6.....      | (120)                       | (108)         | (108)       | (90)         | 90                         |              |                  | 58               |
| May 7.....      |                             | 108           |             | 90           | 96                         | 82           | 72               | 63               |
| May 8.....      | 96                          | 102           |             | 90           | 96                         | 86           |                  | 64               |
| May 13.....     | (126)                       | (102)         | (114)       | (90)         | 90                         | 88           | 70               | 63               |
| May 14.....     | (108)                       | (108)         | (102)       | (96)         | 96                         | 92           | 78               | 64               |
| May 22.....     | (120)                       | (108)         | (102)       | (96)         | 76                         | 90           | 77               | 70               |
| May 23.....     | (108)                       | (114)         | (102)       | (96)         | 90                         | 88           | 78               | 66               |
| May 26.....     | (126)                       | (114)         | (120)       | (114)        | 102                        | 108          | 80               | 73               |
| May 27.....     | 126                         | 120           | 120         |              | 102                        | 98           | 76               | 71               |
| Average I.....  | 129                         | 117           | 126*<br>105 | 90           | } April 18-May 6 inclusive |              |                  |                  |
| Average II..... |                             | 123           |             | 108*<br>97   |                            |              |                  |                  |
| Average I.....  | 117                         | 109           | 108         | 92           | } May 13-26 inclusive      |              |                  |                  |
| Average II..... |                             | 113           |             | 100          |                            |              |                  |                  |

Turning the attention to the *evening pulse rate*, that is the rate at about five hours after the cessation of the exercise, one finds noteworthy data in series '16-cy-ten. If this series be divided into two parts and comparison be made between the average evening pulse rate during the first part (April 14 to May 15 inclusive) with the average evening pulse rate during the second part (May 16 to June 3 inclusive) selecting in both cases the days upon which exercise was performed, the former is

found to be 68.7, the latter 61.4. From this one would conclude either that the effect of training and practice favored the return of the pulse rate to normal, or that the difference of rate of return to normal resulted from the difference in the form of exercise, namely, cycle riding in the first case and tennis in the second.<sup>5</sup> To the writer, the latter alternative seems to be the less important factor.<sup>6</sup>

### RESULTS III

*Effect of training on infection.* The effect of training on the course of an acute naso-pharyngeal infection was noted during the period of observation in 1916. The subject was attacked with the malady in question on April 3 and again on June 8. Both attacks began with nasal and pharyngeal inflammation and marked constitutional symptoms. Then, after a period of rapid convalescence, a laryngitis set in with a return of the constitutional symptoms. This in turn gave place to complete recovery.

On perusal of the blood pressure readings (see table C and fig. 3) the writer cannot see that the blood pressure has been affected at all by either infection, even after the data were charted with reference to the diurnal changes and subjected to close scrutiny no effect could be detected. The pulse rate however shows a great increase during the first attack, but a relatively small one during the second (see fig. 11).

It should be noted that the symptoms in both cases (both local and constitutional) occurred before the change in pulse rate.

The interest in these attacks lies in the facts, *a*, that the observations of blood pressure and pulse rate were begun a considerable time before the infection occurred and were continued for some time after and the abnormal phenomena are therefore carefully controlled; and *b*, that the infections in question were very similar to each other and also to other infections not infrequently experienced by the subject, which run in every case a perfectly definite and predicable course, independent to a

<sup>5</sup> Unfortunately, in the first part of series '14-run observations were rarely made after the end of two minutes, so that a conclusive comparison cannot be made between the evening pulses of the first and second series. This is the more to be regretted since the few values obtained seemed to contradict the conclusions drawn from series '16-cy-ten.

<sup>6</sup> Indeed since it is often found that prolonged exercise favors a retarded return of the pulse to normal, one might expect that, after one and a half to two hours of tennis, the pulse would return to normal more slowly than after the thirty-minute cycle rides.



great degree of such differences in the weather conditions as distinguish early April from early June in Madison in 1916. It seems probable therefore that the only variable factor and hence the cause of any difference between these two attacks is the fact that the subject was in bad physical condition during the first attack while the contrary was the case during the second attack.

*Effect of acute infection on the response of the pulse rate to exercise.* It is of interest and also is germane to the present topic to note at this point the effect of an acute infection upon the response of the pulse rate to exercise in a case of acute naso-pharyngeal affection. The writer has long been familiar with the feeling that the heart beats more rapidly

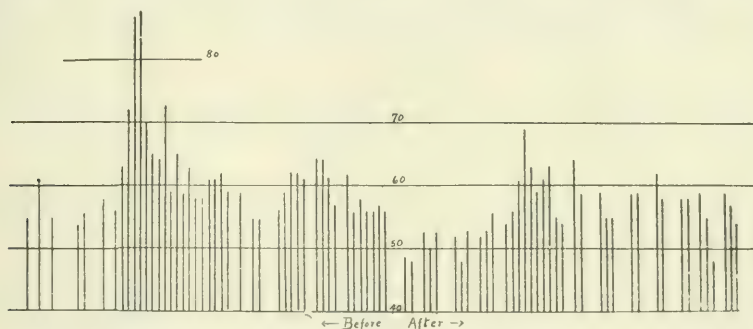


Fig. 11. Effect of training upon the pulse rate during an acute infection. The figures in the ventricular column signify pulse beats per minute. The ventricular lines show the pulse rate in series '16-cy-ten. They are arranged in the order of time. Wherever the observer failed to determine the pulse rate at the appointed hour, a blank space has been left. The observations fall into two groups (1) early in the series (2) late in the series; in other words 'before' and 'after' training. The maximum pulse rate "before" occurs on the evening of the first day (April 3) upon which symptoms of the first infection were observed; that "after" on the evening of the first day (June 8) of the symptoms of the second infection. Note that the maximum before training is much higher than that after training.

on running up stairs when one has a "cold" than under normal circumstances. No careful observations were made until May, 1919. At that time the writer was engaged in a series of experiments which involved turning by hand the wheel of a cycle ergometer having a four kilo break. The mode of procedure was to work for ten minutes and rest for five minutes alternately for the space of an hour. During each five minutes of rest, the pulse was counted. The counting began at exactly fifteen

seconds after the cessation of the exercise and was continued every other quarter-minute until eight counts were made. Thus four minutes elapsed and the remaining minute of rest was devoted to reading the ergometer and in getting ready for the next ten minutes of work.

In the course of these experiments an acute naso-pharyngeal infection occurred and two tests were performed while the subject was "under the weather" (May 17 and 19). On comparing the effect of the muscular exertion upon the heart rate, we find *a*, that the heart rate is much higher during the infection than before while the work done is on the average less; *b*, that the heart rate is no less during the infection than afterward while the work done is much less. The rise in the heart rate after the period of infection as compared with that before infection is the result of practice which permits a great increase

TABLE 10

*Effect of acute infection on the response of the heart rate to exercise*

| DATE              | PULSE RATE         |                 |       |       |       | NUMBER OF REV-<br>OLUTIONS |
|-------------------|--------------------|-----------------|-------|-------|-------|----------------------------|
|                   | Before<br>exercise | After each heat |       |       |       |                            |
|                   |                    | 1st             | 2d    | 3d    | 4th   |                            |
| May 5, 1919.....  | 55                 | 21-15           | 24-16 | 27-17 | 27-19 | 5610                       |
| May 17, 1919..... | 56                 | 29-19           | 28-20 | 30-20 | 30-19 | 5157                       |
| May 19, 1919..... | 53                 | 30-19           | 34-20 | 32-21 | 32-22 | 5992                       |
| June 9, 1919..... | 59                 | 30-20           | 34-22 | 32-21 | 34-23 | 7098                       |

Average

5574

Average  
5574

in the number of revolutions accompanied by an increase in the heart rate without however any untoward effects. But the infection causes increase in the heart rate without permitting any corresponding increase in the amount of work done, and the exertion in this case distresses the subject to a considerable extent.

Table 10. In the *first* column is shown the date of the experiment; in the *second*, the pulse rate per minute before exercise; *third*, the pulse rate after the first ten minutes of work, the first figure representing the first reading, the second figure the last reading in the first four minutes following the first heat; *fourth*, *fifth* and *sixth* observations after the second, third and fourth heats respectively; in the *seventh*, the total number of revolutions in all four heats. May 5 and June 9 are before and after the infection respectively. May 17 and 19 are during the infection. *Note that during infection the reaction of the pulse rate to exercise is increased while the number of revolutions (amount of work done) is not increased.*

*Exercise and distress.* A few notes were made of the feeling of distress which occasionally occurred *during* exercise ('14-run). Here there seemed to be no discoverable connection between these symptoms and the extent of the disturbance of the pulse rate or blood pressure subsequently obtained (at the end of the run) nor between the symptoms and the return of the pulse rate toward normal. For example, on May 22 and April 17 following distress, the pulse rate was 120 and 100 respectively while no untoward symptoms occurred on May 1, 2 and 8 when the pulse rate at the end of the run was 150, 120 and 96 respectively.

*Following* exercise there may be a feeling of distress which consists of giddiness or nausea or both. This does not necessarily occur during the negative phase. It may occur some minutes after the exercise has ceased. It was of momentary duration in every case except after the first bicycle ride in 1914. In the latter case the sequence of events was as follows:

| TIME   | S.  | D. | P.R.         |
|--|-----|----|--------------|
| 5.08 p.m. cessation of exercise                            |     |    |              |
| 5.19   | 116 | 90 | 90           |
| Began to feel faint  |     |    |              |
| 5.20   | 118 | 90 |              |
| 5.21   | 116 | 92 |              |
| Kneeling with head touching floor                          |     |    |              |
| Feeling better, head still low                             |     |    |              |
| 5.25   | 118 | 90 | 52           |
| Head raised, still kneeling                                |     |    |              |
| 5.26   | 118 | 88 | 88           |
| 5.27   | 116 | 88 | 80           |
| 5.28   | 116 | 88 | 126          |
| Sitting on bicycle again                                   |     |    |              |
| 5.30   | 115 | 88 |              |
| 5.35   | 116 | 90 | 126          |
| Subject lies down  |     |    |              |
| 5.43   | 108 | 80 | 76 recumbent |
| 5.48   | 106 | 80 | 82 recumbent |
| Subject changes clothes partly                             |     |    |              |
| 5.57   | 118 | 76 | 76 recumbent |
| Finished dressing  |     |    |              |
| 6.05   | 124 | 80 | 74           |
| Dinner with staff of medical school followed by discussion |     |    |              |
| 10.00  | 115 | 75 | 67 recumbent |
| 10.00  | 118 | 80 | 84 standing  |

A satisfactory interpretation of this attack must await more extensive observation and the following explanation is little more than a conjecture. It appears to the writer as if the primary factors were cardiac. A sudden drop in the pulse rate (from 90 to 52) causes cerebral and systemic anemia, a powerful, peripheral constriction restores the blood pressure (reading B.P. 118-90, P.R. 52). Then the peripheral constriction all the time compensating the changes in pulse rate, the latter rises, runs beyond the mark, falls again, rises again, and finally becomes tranquil. It is, of course, possible to conceive that the vascular change (constriction) is primary and is compensated by the changes in heart rate. But such a supposition seems unwarranted since it implies that the cerebral vessels share in the general constriction to a degree that renders the brain anemic, an exploit on the part of cerebral vasomotors which at the present time seems incredible.

Lastly it might be urged that perhaps the factor of peripheral resistance remains constant and that the factor which varies inversely with the heart rate (thus keeping the pressure constant) is the force (output) of the heart beat. But the writer is still addicted to the belief that such variations in systolic output are indicated by change in the pulse pressure and, since this does not occur, he is loath to entertain this explanation.

During the vicissitudes of the experiment Professor Eyster made the determinations for which I am indebted.

In 1916, distress was sometimes observed after cycle rides. On one occasion, April 14, fleeting faintness was felt and on the other, May 3, the symptom was an equally transitory nausea. On neither occasion did these symptoms last longer than the time required for a single pair of blood pressure readings. The faintness was not associated with any decrease in pulse pressure though the nausea may have been (fig. 10). Unfortunately, the pulse rate was not determined in these particular experiments. The symptoms on these two occasions differ from those in 1914, not only in being less severe but also in their time relations to the cessation of the exercise. Those of '16-cy-ten were within five minutes of the cessation of exercise (cycle) while that in '14-run was fifteen minutes after cessation of the exercise (cycle test).



## SUMMARY OF RESULTS

The following summary is submitted without further discussion. Credit to previous investigators together with comment and criticism is reserved for a separate communication.

I. The effect of training upon the resting pulse rate, blood pressures and their derivatives was as follows:

*a.* The pulse rate was slowed especially the noon and afternoon pulses. This altered slightly the form of the diurnal pulse curve. The extent of the diurnal variation was increased.

*b.* The diurnal variations of the systolic pressure were increased.

*c.* The diastolic pressure approached the daily mean while its diurnal variations were much decreased.

*d.* The pulse pressure was usually increased and its diurnal variations were somewhat decreased.

*e.* The forms of the daily curves of blood pressures were not obviously altered.

*f.* The product of the pulse rate times the pulse pressure was usually increased but the character of its daily variations was unchanged.

II. The effect of training or of practice or of both upon the cardiovascular reactions to exercise was as follows:

*g.* When the trained or practiced individual engaged in physical exercise he naturally accomplished more work with less apparent exertion and less subjective distress. This was in spite of the fact that the systolic pressure rose higher, the pulse pressure increased enormously and with it sometimes the product  $P.R. \times P.P.$

*h.* The diastolic pressure sometimes returned earlier to normal, i.e., while the exercise was still in progress.

*i.* The pulse rate however was sometimes less affected than in the untrained subject and probably reached normal before that of the latter.

III. The following interesting, miscellaneous observations were also made:

*j.* An acute infection (nasopharyngitis) caused an increase in the pulse rate but no change in the blood pressures. In the trained subject the change in pulse rate was much less pronounced.

*k.* During acute infection (nasopharyngitis) exercise caused a greater increase in pulse rate than with the normal subject and the amount of work accomplished was less.

l. The feeling of distress which occurred during exercise showed no relation to the heart rate and blood pressure determined at the cessation of the exercise. When distress followed exercise it had no relation to the blood pressure present at the time but the heart rate was found to be greatly decreased.

As already stated in the introduction (q.v.), these conclusions are subject to certain reservations.

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## APPENDIX. ROUTINE OBSERVATIONS

TABLE A

*14-run*

| DATE         | MORNING |      | NOON    |      | AFTERNOON |      | EVENING |      | REMARKS               |
|--------------|---------|------|---------|------|-----------|------|---------|------|-----------------------|
|              | S-D.    | P.R. | S-D.    | P.R. | S-D.      | P.R. | S-D.    | P.R. |                       |
| April 1....  |         |      |         |      |           |      |         |      | Cycle test            |
| April 2....  |         |      | 116-88  | 50   | 120-98    | 59   |         |      |                       |
| April 3....  |         |      | 120-104 | 55   | 128-100   | 61   |         |      |                       |
| April 4....  |         |      | 124-90  | 67   | 122-92    | 62   |         |      |                       |
| April 5....  |         |      | 122-92  | 52   | .         |      |         |      | 8 miles, walk and run |
| April 6....  |         |      |         |      |           |      |         |      | 8 miles, walk and run |
| April 7....  |         |      |         |      |           |      |         |      | 8 miles, walk and run |
| April 8....  |         |      |         |      |           |      |         |      | 3 miles, walk and run |
| April 9....  |         |      | 130-98  | 58   |           |      |         |      | 8 miles, walk and run |
| April 10.... |         |      |         |      |           |      |         |      | Gymnastics            |
| April 11.... |         |      |         |      |           |      |         |      | 12-mile walk          |
| April 12.... | 118-86  |      |         |      | 126-92    | 82   |         |      | 22-mile walk          |
| April 13.... |         |      | 116-82  | 50   |           |      |         |      | Gymnastics            |
| April 14.... |         | 54   | 124-90  | 56   | 114-90    | 64   |         |      |                       |
| April 15.... |         | 54   |         |      |           |      |         |      | 3 miles, walk and run |
| April 16.... |         |      | 120-86  | 64   | 120-82    | 60   |         |      |                       |
| April 17.... |         |      |         |      | 128-90    | 58   |         |      | 3 miles, walk and run |
| April 18.... |         |      |         |      |           |      |         |      | 3 miles, walk and run |
| April 19.... |         |      |         |      |           |      |         |      | Coryza                |
| April 22.... |         |      |         |      |           |      |         |      | 3-mile run            |
| April 23.... |         |      |         |      |           |      |         |      |                       |
| April 24.... |         | 54   |         |      |           |      |         |      |                       |
| April 25.... |         |      | 122-88  | 56   |           |      |         |      |                       |
| April 26.... |         |      |         |      |           |      |         |      |                       |
| April 27.... |         |      |         |      |           |      |         |      | 3-mile run            |
|              | *       | *    | *       | *    | *         | *    | *       | *    |                       |
| April 30.... |         |      |         |      |           |      |         |      | 3-mile run            |
| May 1.....   |         |      |         |      |           |      |         |      | 3-mile run            |
| May 2.....   |         |      |         |      |           |      | 128-88  | 56   | 3-mile run            |
| May 3.....   | 111-85  | 54   |         |      |           |      | 96-74   | 59   | 13-mile walk          |
| May 4.....   | 112-81  | 52   |         |      |           |      |         |      |                       |
| May 5.....   |         | 53   |         |      |           |      |         |      |                       |
| May 6.....   |         |      |         |      | 109-77    | 53   | 110-77  | 58   | 3-mile run            |
| May 7.....   | 115-80  | 47   |         |      | 126-83    | 55   | 115-81  | 63   | 3-mile run            |
| May 8.....   | 110-76  | 55   |         |      | 113-78    | 55   |         |      | 3-mile run            |
| May 9.....   |         |      | 120-94  | 50   | 120-94    | 52   |         |      |                       |
| May 10....   |         |      | 114-87  | 51   |           |      |         |      |                       |
|              | *       | *    | *       | *    | *         | *    | *       | *    |                       |

TABLE A—*Concluded*

| DATE        | MORNING |       | NOON   |       | AFTERNOON |       | EVENING |       | REMARKS    |
|-------------|---------|-------|--------|-------|-----------|-------|---------|-------|------------|
|             | Z. D.   | P. R. | Z. D.  | P. R. | S. D.     | P. R. | S. D.   | P. R. |            |
| May 13..... |         |       |        |       | 113-83    | 59    | 105-75  | 63    | 3-mile run |
| May 14..... | 107-85  | 58    |        |       | 120-87    | 63    | 108-85  | 64    | 3-mile run |
| May 15..... |         | 55    |        |       |           |       |         |       |            |
| May 22..... |         |       |        |       | 114-84    | 53    | 105-84  | 70    | 3-mile run |
| May 23..... | 100-81  | 50    | 114-88 | 53    | 115-86    | 57    | 130-90  | 66    | 3-mile run |
| May 24..... | 109-89  | 57    | 112-83 | 53    | 110-83    | 54    | 122-84  | 54    |            |
| May 25..... | 106-81  | 48    | 112-91 | 52    |           |       | 119-90  | 68    |            |
| May 26..... | 116-83  | 55    | 119-89 | 61    | 120-91    | 62    | 108-79  | 73    | 3-mile run |
| May 27..... | 101-81  | 53    |        |       | 121-87    | 64    | 103-83  | 71    | 3-mile run |
| May 28..... | 101-78  | 52    | 122-92 | 54    | 123-94    | 57    | 115-89  | 54    |            |
| May 29..... | 108-89  | 49    |        |       | 117-90    | 52    | 119-95  | 55    | Cycle test |
| May 30..... | 106-82  | 50    | 117-85 | 48    |           |       |         | 57    |            |
| May 31..... |         | 47    |        | 56    |           | 53    |         | 51    |            |
| June 1..... |         | 54    |        | 51    |           | 53    |         | 58    |            |
| June 2..... |         | 49    |        | 49    |           | 53    |         |       |            |



TABLE B

\* 15-gm.

| DATE           | MORNING |      | NOON   |      | AFTERNOON |      | EVENING |      |
|----------------|---------|------|--------|------|-----------|------|---------|------|
|                | S.-D.   | P.R. | S.-D.  | P.R. | S.-D.     | P.R. | S.-D.   | P.R. |
| March 5.....   |         |      | 112-88 | 64   | 118-84    | 60   | 111-85  | 59   |
| March 6.....   | 109-82  | 57   | 107-82 | 58   |           |      |         |      |
| March 7.....   |         |      | 109-78 | 63   |           |      | 118-80  | 67   |
| March 8.....   | 103-90  | 54   | 110-81 | 60   | 114-85    | 60   | 113-88  | 59   |
| March 9.....   | 113-88  | 56   | 111-91 | 61   |           |      | 110-79  | 63   |
| March 10.....  | 114-90  | 52   |        |      | 113-89    | 62   |         |      |
| March 11.....  | 114-86  | 54   | 126-85 | 61   | 118-91    | 58   | 118-86  | 62   |
| March 12.....  | 112-85  | 58   | 110-89 | 60   |           |      |         |      |
| March 13*..... | 109-77  | 69   | 114-91 | 55   |           |      | 114-78  | 60   |
| March 14*..... | 105-76  | 70   | 112-77 | 59   |           |      | 114-78  | 60   |
| March 15*..... | 108-77  | 72   | 108-79 | 61   | 120-90    | 63   | 117-86  | 64   |
| March 16*..... | 114-78  | 71   |        |      |           |      | 113-87  | 59   |
| March 17*..... | 108-75  | 69   | 117-86 | 69   | 113-80    | 64   | 116-86  | 60   |
| March 18*..... | 110-76  | 70   |        |      |           |      | 108-80  | 58   |
|                | *       | *    | *      | *    | *         | *    | *       | *    |
| March 21*..... | 105-70  | 75   |        |      |           |      | 114-76  | 70   |
| March 22*..... | 113-76  | 74   | 116-88 | 62   | 116-87    | 63   | 117-81  | 66   |
| March 23*..... | 109-76  | 67   |        |      | 119-86    | 58   |         |      |
| March 24*..... |         | 68   | 115-89 | 53   |           |      |         |      |
| March 25*..... | 111-76  | 60   |        |      |           |      | 122-85  | 65   |
| March 26*..... | 116-84  | 66   | 116-92 | 60   |           |      |         |      |
| March 27*..... | 111-77  | 72   | 115-88 | 65   |           |      | 115-84  | 72   |
| March 28.....  | 115-78  | 57   | 112-86 | 57   |           |      | 123-81  | 61   |
| March 29*..... | 115-84  | 68   | 119-89 | 58   |           |      | 118-80  | 61   |
| March 30*..... | 109-79  |      | 114-87 | 56   |           |      | 125-79  | 70   |
| March 31*..... | 112-74  | 70   |        |      | 112-85    | 56   | 129-85  |      |
| April 1.....   | 111-77  | 53   | 117-85 | 53   | 118-85    | 56   | 120-86  | 55   |
| April 2*.....  | 118-78  | 65   | 124-86 | 63   | 121-85    | 60   |         |      |
| April 3*.....  | 111-79  | 69   |        |      | 118-87    | 62   |         |      |
| April 4.....   | 117-84  | 51   |        |      |           |      | 117-75  | 67   |
| April 5*.....  | 104-68  | 74   |        |      | 114-86    | 70   | 120-83  | 63   |
| April 6*.....  | 111-75  | 65   | 113-82 | 60   | 118-89    | 56   | 120-95  | 62   |
| April 7.....   | 113-82  | 53   |        |      | 114-87    | 55   |         |      |
| April 8*.....  | 111-75  | 67   | 107-83 | 56   | 115-87    | 60   | 121-82  | 60   |
| April 9*.....  | 107-73  | 64   | 114-88 | 55   | 115-85    | 59   | 108-75  | 60   |
| April 10†..... | 104-68  | 62   | 119-93 | 51   | 117-85    | 63   |         | 54   |
| April 11.....  |         |      |        |      |           |      |         |      |
| April 12.....  | 107-72  | 69   | 130-92 | 57   |           |      | 109-79  | 63   |
| April 13*..... | 105-77  | 73   |        |      |           |      |         |      |
| April 14.....  |         | 50   | 121-95 |      | 120-89    |      | 116-79  | 58   |
| April 15.....  | 110-76  | 52   | 121-95 | 50   | 127-95    | 65   | 115-78  | 59   |
| April 16.....  | 112-81  | 55   |        |      |           |      |         |      |
| April 17.....  | 110-80  | 55   | 122-88 |      |           |      |         |      |
| April 18.....  |         | 56   |        |      |           |      |         |      |
| April 19.....  |         |      | 124-91 | 60   | 126-93    | 62   |         |      |

\* Gymnastics on rising before making observations of pressure and pulse.

† Gymnastics in afternoon before observations.

TABLE C  
*'16-cy-ten*

| DATE          | MORNING |       | NOON   |       | AFTERNOON |       | EVENING |       | REMARKS      |
|---------------|---------|-------|--------|-------|-----------|-------|---------|-------|--------------|
|               | S. D.   | P. R. | S. D.  | P. R. | S. D.     | P. R. | S. D.   | P. R. |              |
| March 30..... |         | 55    |        |       |           | 62    |         |       |              |
| March 31..... |         | 55    |        |       |           |       |         |       |              |
| April 1.....  | 101-67  | 55    | 118-75 | 66    |           |       |         |       |              |
| April 2.....  | 101-73  | 58    |        |       | 118-82    | 56    | 120-81  | 65    |              |
| April 3.....  |         | 63    |        |       | 126-83    | 85    | 118-73  | 86    |              |
| April 4.....  | 106-72  | 70    | 108-80 | 65    | 120-84    | 64    | 104-70  | 73    |              |
| April 5.....  | 109-72  | 57    | 113-86 | 65    | 117-86    | 57    | 107-79  | 63    |              |
| April 6.....  | 98-71   | 58    | 119-86 | 58    | 119-90    | 61    | 103-72  | 62    |              |
| April 7.....  | 99-73   | 59    |        |       | 115-78    | 59    |         |       |              |
| April 8.....  | 103-76  | 57    | 119-90 | 59    |           |       | 124-85  | 62    |              |
| April 9.....  | 103-73  | 61    |        |       | 106-78    | 66    | 120-90  | 66    | Walk         |
| April 10..... | 100-76  | 61    | 110-81 | 57    | 118-89    |       | 115-83  | 62    |              |
| April 11..... | 109-81  | 56    | 111-84 | 58    | 118-83    | 56    | 116-80  | 56    |              |
| April 12..... | 100-72  | 57    | 110-80 | 56    |           |       | 114-79  | 79    | Walk         |
| April 13..... | 101-79  | 57    | 114-84 | 55    | 114-84    | 55    | 121-88  | 58    |              |
| April 14..... | 106-85  | 60    | 112-82 | 59    | 116-79    | 66    | 107-76  | 66    | Cycle ride   |
| April 15..... | 100-77  | 56    | 116-90 | 60    | 111-87    | 54    | 112-79  | 65    | Walk         |
| April 16..... | 97-71   | 54    | 109-79 | 56    | 116-78    | 65    | 98-68   | 70    | Cycle ride   |
| April 17..... | 101-74  | 50    | 120-91 | 58    | 119-85    | 64    | 115-76  | 60    |              |
| April 18..... | 103-76  | 56    | 121-91 | 56    | 115-79    | 60    | 107-78  | 63    | Cycle ride   |
| April 19..... | 99-68   | 54    | 116-87 | 66    | 117-85    | 64    | 102-75  | 65    |              |
| April 20..... | 96-64   | 56    | 106-78 | 66    | 111-82    | 60    | 112-76  | 70    |              |
| April 21..... | 102-74  | 55    |        |       | 116-81    | 62    | 108-73  | 68    | Cycle ride   |
| April 22..... | 105-70  | 53    | 114-80 | 64    |           |       | 116-81  | 65    |              |
| April 23..... | 106-81  | 56    | 112-83 | 54    |           |       | 111-81  | 68    | 6-mile walk  |
| April 24..... | 100-77  | 56    |        |       | 110-80    | 72    |         |       |              |
| April 25..... |         | 55    |        |       |           |       | 116-78  | 65    |              |
| April 26..... | 107-77  | 59    |        |       |           |       |         |       |              |
| April 27..... | 112-78  | 52    |        |       | 115-84    | 64    | 114-80  | 68    | Cycle ride   |
| April 28..... | 102-72  | 54    | 109-81 | 56    | 116-88    | 59    | 100-70  | 62    |              |
| April 29..... | 106-84  | 53    | 112-85 | 58    | 111-84    | 56    |         |       | Cycle ride   |
| April 30..... | 107-83  | 53    | 106-80 | 59    | 113-80    | 58    | 100-79  | 56    |              |
| May 1.....    | 100-79  | 56    |        |       |           |       |         |       |              |
| May 2.....    | 102-76  | 63    | 112-86 | 62    | 112-82    | 62    | 116-81  | 64    |              |
| May 3.....    | 106-77  | 58    | 115-83 | 62    | 123-91    | 66    | 109-80  | 69    | Cycle ride   |
| May 4.....    | 105-78  | 57    |        |       | 56        |       | 119-84  | 58    |              |
| May 5.....    | 104-80  | 57    | 115-90 |       |           |       | 113-84  | 74    | Tennis, walk |
| May 6.....    | 101-78  | 60    | 116-84 | 61    | 111-82    | 61    | 126-85  | 64    |              |
| May 7.....    | 108-78  | 57    | 109-81 | 55    | 111-77    | 68    | 110-80  | 72    | Cycle ride   |
| May 8.....    | 104-81  | 54    | 111-84 | 59    | 113-81    | 61    | 112-80  | 61    |              |

TABLE C—*Concluded*

| DATE         | MORNING |      | NOON |        | AFTERNOON |        | EVENING |        | REMARKS    |
|--------------|---------|------|------|--------|-----------|--------|---------|--------|------------|
|              | S.D.    | P.R. | S.D. | P.R.   | S.D.      | P.R.   | S.D.    | P.R.   |            |
| May 9.....   | 103     | 86   | 55   | 116-86 | 59        |        |         |        | Tennis     |
| May 10.....  | 105     | 83   | 58   | 113-84 | 56        | 115-82 | 58      |        |            |
| May 11.....  | 107     | 77   | 56   | 108-84 | 53        |        |         |        | Tennis     |
| May 12.....  | 107     | 76   | 54   | 115-86 | 57        |        |         |        |            |
| May 13.....  | 102     | 76   | 56   | 112-89 | 55        |        | 114-83  | 59     |            |
| May 14.....  | 107     | 79   | 53   |        | 64        |        | 113-80  | 64     |            |
| May 15.....  | 100     | 77   | 58   | 108-80 | 62        |        | 109-85  | 69     | Tennis     |
| May 16.....  | 108     | 78   | 55   | 112-84 | 53        |        | 107-82  | 69     | Tennis     |
| May 17.....  | 106     | 78   | 55   | 119 87 | 55        | 111 84 | 62      | 111-81 | 62         |
| May 18.....  | 108     | 80   | 57   | 109-81 | 56        |        | 115-79  | 64     | Tennis     |
| May 19.....  | 104     | 71   | 54   | 111-84 | 57        | 116-83 | 60      | 110-80 | 64         |
| May 20.....  | 113     | 77   | 59   | 113-83 | 60        | 114-85 | 59      | 116-84 | 61         |
| May 21.....  | 112     | 79   | 51   |        |           |        | 122-81  | 64     |            |
| May 22.....  | 110     | 78   | 55   | 115-82 | 52        | 117-84 | 62      | 104-73 | 72         |
| May 23.....  | 110     | 78   | 61   | 110-83 |           |        | 107-78  | 73     | Tennis     |
| May 24.....  | 108     | 77   | 59   | 110-77 | 52        | 121-84 | 60      |        |            |
| May 25.....  |         |      |      | 114-85 | 56        |        | 107-80  | 58     | Tennis     |
| May 26.....  | 106     | 81   | 55   | 120-84 | 60        |        | 112-79  | 58     | Tennis     |
| May 27.....  | 105     | 72   | 53   | 112-80 | 51        |        | 110-77  | 64     | Tennis     |
| May 28.....  | 102     | 71   | 56   | 113-81 | 47        |        | 60      | 115-82 | 60         |
| May 29.....  | 112     | 82   | 48   | 109-83 | 47        | 117-86 | 49      | 115-77 | 56         |
| May 30.....  | 106     | 79   | 52   | 112-83 | 50        |        | 111-81  | 58     | Tennis     |
| May 31.....  | 105     | 76   | 53   | 114-84 | 50        | 109-82 | 53      | 116-76 | 56         |
| June 1.....  |         |      | 53   | 114-82 | 50        |        |         |        | Tennis     |
| June 2.....  | 105     | 79   | 58   | 113-81 | 58        |        |         |        | Tennis     |
| June 3.....  | 98      | 77   | 54   | 112-79 | 48        | 115-77 | 59      | 115-77 | 59         |
| June 4.....  | 105     | 78   | 49   | 107-81 | 48        |        | 116-80  | 53     |            |
| June 5.....  | 108     | 80   | 50   | 106-77 | 53        |        |         |        |            |
| June 6.....  | 108     | 80   | 52   | 115-83 | 48        | 115-80 | 53      |        | Cycle ride |
| June 7.....  | 112     | 74   | 52   | 112-83 | 53        | 116-79 | 56      |        | Cycle ride |
| June 8.....  | 110     | 80   | 51   | 118-88 | 56        | 119-86 | 61      | 112-78 | 69         |
| June 9.....  | 115     | 79   | 63   | 118-81 | 59        | 116-83 | 61      | 118-77 | 63         |
| June 10..... | 106     | 77   | 55   | 115-81 | 54        |        | 115-78  | 66     |            |
| June 11..... | 99      | 70   | 59   | 110-80 | 53        | 117-81 | 56      | 119-76 | 59         |
| June 12..... | 101     | 76   | 55   | 120-83 | 55        |        |         |        | Tennis     |
| June 13..... | 107     | 77   | 59   | 112-79 | 59        |        | 113-80  | 70     | Tennis     |
| June 14..... | 110     | 77   | 62   | 112-79 | 58        |        |         |        | Tennis     |
| June 15..... | 107     | 76   | 58   | 115-85 | 57        |        | 108-78  | 59     |            |
| June 16..... | 102     | 79   | 55   | 112-80 | 48        |        | 116-80  | 59     |            |
| June 17..... | 104     | 78   | 54   | 118-83 | 50        |        |         |        |            |





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## CARDIO-VASCULAR REACTION IN THE VALSALVA EXPERIMENT AND IN LIFTING WITH A NOTE ON PARTURITION<sup>1</sup>

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### INTRODUCTION

The object of the following experiments was to determine the immediate effect upon the heart rate and blood pressures of exercises of strain. By "exercises of strain" is meant those muscular efforts which are performed with the glottis closed and which tend to compress the chest, thereby causing a rise in intrathoracic pressure. The two forms of strain studied were the Valsalva experiment and lifting.

The importance of the subject may be realized when we remember that the conditions which obtain during lifting and the Valsalva exper-

<sup>1</sup> A preliminary report of this work was made at the annual meeting of the American Physiological Society and was published in this Journal (2).

<sup>2</sup> It has seemed to me no more than just to place the name of Doctor Hodges where it stands. I am, however, reluctant to make him responsible for statements, perhaps erroneous, which are not his and in regard to which I have not consulted him owing to his absence in China. Consequently I offer this word of explanation.

In 1914-1915 Doctor Hodges and I worked upon the Valsalva experiment and our results appeared in his Bachelor's thesis (3) and in a preliminary report presented by me (2). Subsequently I continued and amplified this work considerably and entirely rearranged such parts of the thesis and preliminary report as I wished to embody in the final communication.

I think it would have been absurdly awkward to have presented separately our work and mine.—P. M. D.

iment are very similar to those which occur in difficult defecation, parturition and severe cough.<sup>3</sup>

In this article will be found a consideration *a*, of the Valsalva experiment; *b*, of lifting; *c*, of certain modifications which may be applied to these two maneuvers; and lastly, *d*, of an interesting observation on a rabbit during labor reflexly induced.

#### THE VALSALVA EXPERIMENT

The Valsalva experiment consists in a forced expiratory movement with the glottis closed. The effectiveness of the expiratory effort may be enhanced by taking a deep breath just before the forced expiration has begun and also by raising the knees and pressing the thighs firmly against the abdomen, so as to support the abdominal walls during the expiratory movement. Usually the effort was continued as long as possible, that is, until the respiratory distress became excessively disagreeable.

A discussion of the phenomena observed during the Valsalva experiment falls naturally into three parts which concern the blood pressure, the pulse rate and the pulse form respectively. This separation is not only on the basis of subject matter but also on that of method of experimentation, the sphygmomanometer, the string galvanometer and the sphygmograph being the instruments respectively employed in each part of this study. The sphygmomanometer was that of Erlanger which was connected with a tank of compressed air by means of which the cuff could be inflated more rapidly than with the bulb as ordinarily employed. The graphic method was supplemented by the auscultatory, use being made of whichever method seemed the more suitable to the special occasion. The sphygmograph was that of Dudgeon, modified as suggested by Lewis (4) by the substitution of freely hanging weights for the straps which ordinarily secure the instrument to the wrist.

#### *Blood pressure (systolic)*

1. *Primary rise (of McCurdy) (6).* The cuff was strapped about the subject's arm, with the stethoscope below, and the normal systolic pressure determined by the auscultatory method. Air was then allowed to enter the cuff rapidly until the mercury stood at 180 mm.;

<sup>3</sup> The history of the Valsalva experiment has seemed so interesting to one of us (D) that he purposes to present it in a separate communication.

at this point the subject was instructed to fill his lungs and strain with closed glottis. If the resulting rise in blood pressure surpassed 180, it caused the pulse to "break through" beneath the cuff, producing a sound which the observer could hear with the stethoscope. The time elapsing between the beginning of the effort and the appearance of this sound, together with the total duration of the effort, were recorded by means of a stop watch. In this way it was possible to determine at what stage of the effort the blood pressure reached 180 mm. Hg., and, by repeating the procedure with increasing pressures in the cuff, it was possible to find the maximum height to which the blood pressure rose, that is, the pressure in the cuff beyond which the pulse failed to "break through."

The following protocols of April 1, 1915, are typical:

*Series I:* subject Dawson; normal systolic pressure 122 (two concordant observations); with pressure in cuff 180 mm., one pulse beat was heard a few seconds after the beginning of the expiratory effort; with pressure in cuff 190 mm. several pulse beats were heard; when a second trial was made with pressure in cuff 180 mm., several pulse beats were heard; at 190 mm., none.

*Series II:* Subject P. C. Hodges; normal systolic pressure 112 (average of several closely agreeing observations); with pressure in cuff 130 mm., two pulse beats were heard; at 140 mm., one beat; at 156, six; 172, three; 180, three; 190, none.

Six subjects thus examined gave primary blood pressure rises of from 62 to 90 mm. Hg., the greatest total pressures being between 180 and 200. The average time elapsing between the beginning of the effort and the height of the rise was about three seconds, the duration of the rise being very short.

2. *Fall (of Valsalva and Weber)* (7). Following the primary rise of systolic pressure there is a very considerable fall due without doubt to the decreased inflow of blood to the heart resulting from the high intrathoracic pressure. Two methods were employed in estimating the extent of this fall. Though neither was such as to establish precisely the quantitative value of the fall, both were highly suggestive.

*First method.* Five subjects were examined, but since the results coincided very closely, a description of the one case will serve for all. The cuff and stethoscope were adjusted as usual, and the pressure raised to 60 mm. At this point the beating of the pulse could be plainly heard. Eight seconds after the beginning of the effort the sound failed, and could not be heard again, though the pressure in the cuff was quickly raised and lowered between 30 and 150 mm. through-

out the rest of the exercise. With expiration the sound promptly reappeared, and the pressure soon returned to normal. The fact that the sound failed with the pressure in the cuff at 60 mm. points to the assumption that either both systolic and diastolic pressures were above 60 mm. during that part of the exercise, or that they had both fallen below 60 mm. and that neither returned to that point until expiration. The first of the hypotheses is excluded by the fact that no beating could be heard when the pressure in the cuff was raised above 60, and we have left only the assumption that there was an actual fall below 60 mm.

These relations are seen in the following table (table 1) which shows the results of three experiments performed on April 27, 1915.

TABLE 1  
*Effect of Valsalva experiments on systolic blood pressure; the fall*

| SUBJECT                 | P. M. D.    | P. M. D.     | P. C. H.     |
|-------------------------|-------------|--------------|--------------|
| Systolic pressure.....  | 116         |              | 108          |
| Pressure in cuff.....   | 90          | 60           | 90           |
| Pulse disappears.....   | 1.6 seconds | 14.8 seconds | 10.8 seconds |
| Pulse reappears.....    | 7.3 seconds | 29.9 seconds | 33.0 seconds |
| Duration of effort..... |             |              | 28.0 seconds |

The time of disappearance and reappearance of the pulse is counted from the beginning of the expiratory effort.

*Second method:* The cuff of the Erlanger sphygmomanometer was adjusted and inflated until it stood (at various levels at different times) below the diastolic pressure. At each level the Valsalva maneuver was performed.

The following protocol is typical:

April, 1914; Dawson subject; Professors Eyster and Meek observers; blood pressures of subject while sitting on stool with knees drawn up and body bent forward 110 to 85 mm. At a given signal a long breath was taken followed by compression of the abdomen against the thighs and forced expiration with effort against the closed glottis. The lever ceased to show pulsations when the pressure in the cuff stood at 70 mm. A second trial was made with the pressure at 60 mm. This time pulsation became feeble and disappeared altogether when the straining was increased a little.

From this it is inferred that the systolic pressure had fallen below 60 mm. though how much below it was impossible to say. It should be remembered that the minimum movements of the lever do not



record systolic pressure but occur when the pressure in the cuff is considerably above systolic pressure. It would seem therefore that the systolic pressure was certainly somewhat below, perhaps considerably below 60 mm. It may be noted in passing that the heart sounds could not be heard with a stethoscope applied to the chest wall during the height of these experiments.

On the cessation of the effort the lever almost immediately began to move and the returning pulse wave soon showed the separation of the ana- and catacrotic limbs announcing that the systolic pressure had become equal to that in the arm cuff.

*Secondary rise* (1). It has been stated that after the fall of pressure described in the preceding section, a rise begins and before the end of the expiratory effort attains a considerable magnitude, as much even as 200 mm. Hg. The writers were unable to verify this assertion. It is true a rise above normal may occur, but we have not found it more extensive than that produced by simply holding the breath which seems to justify the assumption that our secondary rise is only a manifestation of asphyxia. In testing this matter the subject was required to perform the following maneuvers separately: *a*, the Valsalva experiment, the effort being either moderate or severe; *b*, holding the breath with the thorax in the expiratory position; and *c*, the same with chest in a position of very moderate expansion. The negative results are shown in the accompanying table (table 2).

Feeling reluctant to leave the matter of the secondary rise in this unsatisfactory condition, an effort was made to draw further light from animal experiments. The mode of procedure was to increase the intrathoracic pressure in dogs by sudden inflation of the lungs with compressed air. In a typical experiment this resulted in a rise followed by a fall of the mean blood pressure as indicated by the mercury manometer connected with the carotid artery. The rise was slight and brief (8 mm. in 5 sec. from the beginning of the inflation); the fall was extensive and prolonged (92 mm. in 73 sec.). Then because of Bruck's contention that the secondary rise is dependent upon an excess of abdominal over intrathoracic pressure, the abdomen was violently compressed by hand so that the mean blood pressure rose considerably (60 mm.); on relaxing the pressure on the abdomen the mean blood pressure fell, but not quite so low as before. After repeating the maneuver three times the dog became violently dyspneic and the mean blood pressure rose until it was nearly equal to the initial pressure (less by 15 mm.). The distention was then discontinued and the

pressure rose above normal but not much above normal (6 mm.). The accompanying kymogram (fig. 1) shows these relations in this particular experiment.

In a second experiment the blood pressure rose rapidly from normal (150) to 161 mm. Hg., then fell to 98 mm., and rose slowly until it reached 124 mm. Hg. Immediately after the pressure in the lungs was

TABLE 2

*Effects of Valsalva experiment and of simple holding of the breath upon systolic pressure in three subjects*

| NUM-<br>BER OF<br>EX-<br>PERI-<br>MENT | SUBJECT  | SYSTO-<br>LIC BE-<br>FORE<br>EXPERI-<br>MENT | SYSTOLIC PRESSURE<br>DURING EXPERIMENT | DURATION OF<br>EXPERIMENT | KIND OF MANEUVER         |
|--|----------|--|--|---------------------------|--------------------------|
|  |          |  |  | <i>seconds</i>            |                          |
| 1                                      | P. M. D. | 109  | 118, 140                               | 20                        | Valsalva severe          |
| 2                                      | P. M. D. |  | 120, 140, 140                          | 40                        | Valsalva severe          |
| 3                                      | P. M. D. | 108  | 140                                    | 60                        | Valsalva moderate        |
| 4                                      | P. M. D. |  | 140                                    | 100                       | Breath held, expiratory  |
| 5                                      | P. C. H. | 110  | 130, 130                               | 35, 40                    | Valsalva moderate        |
| 6                                      | F. J. H. | 100  | 126, 130                               | 70                        | Valsalva severe          |
| 7                                      | P. M. D. | 112  | 125, 125, 125, 130                     | 100                       | Valsalva severe          |
| 8                                      | P. M. D. |  | 130                                    | 25                        | Breath held, expiratory  |
| 9                                      | F. J. H. |  | 130                                    | 25                        | Breath held, inspiratory |
| 10                                     | F. J. H. |  | 130                                    | 25                        | Breath held, inspiratory |
| 11                                     | F. J. H. | 112  | 120                                    | 20                        | Breath held, expiratory  |
| 12                                     | P. C. H. | 109  | 130, 106, 118                          | 65                        | Valsalva severe          |
| 13                                     | P. C. H. |  | 100, 96, 100                           | 15, 40, 60                | Valsalva severe          |

The figures 100, 96, 100 and 15, 40, 60 given for experiment 13 indicate successive determinations of systolic pressure and the times at which they were respectively made in a single Valsalva effort. The same method of presentation is followed in several of the other experiments in this table. Frequently only the total duration of the effort is noted although several determinations of systolic pressure were made, e.g., experiment 7. *Note that during the effort there is no rise in systolic pressure comparable to that described by Bruck, e.g., 160 to 200 mm.*

released, the blood pressure rose to 168 mm. Hg. From this point it gradually returned to normal.<sup>4</sup>

The writers are therefore still uncertain how to obtain the secondary rise described by Bruck, although a suggestion is offered below (p. 505).

<sup>4</sup> The writers wish to acknowledge the assistance of Dr. Clarence J. Brown in these animal experiments.

*The pulse rate*

During the height of an energetic Valsalva experiment, the radial pulse becomes imperceptible (Valsalva) and upon auscultation the heart sounds are found to be very indistinct or even inaudible (E. H. Weber). It is therefore often impossible to determine the heart rate by either palpation or auscultation and consequently the string-galvanometer was resorted to. The electrodes (large lamp wicks saturated with physiological salt solution) were bound about the forearms. A short control record was made and then at a word of command the subject began to strain and simultaneously a mark was placed upon the record. When the subject desired to cease from his effort, he did so with a loud vocal sound and the observer at once recorded upon the electrocardiogram. The electrocardiographic clock-work continued to run for a short time after the cessation of the effort. It was then stopped but after an interval of thirty seconds was started again for the purpose of making a final observation.

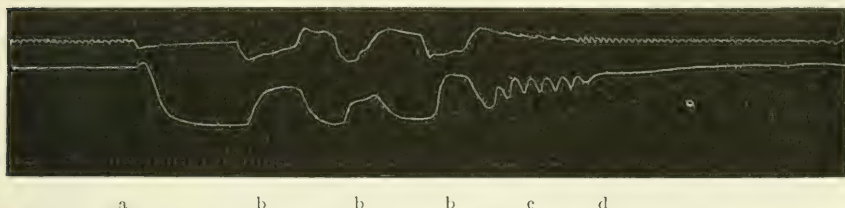


Fig. 1. Effect of inflating lungs of dog with compressed air and of abdominal compression. The *uppermost line* shows the respiratory rate and amplitude as recorded by means of a drum-shaped pneumograph fastened about the body of the animal by an inelastic band. On inspiration the heads of the drum were pulled out. The pneumograph was connected with a recording tambour. Down strokes are inspirations. The extensive variations of this line which occur during the period of inflation of the lungs are merely mechanical, due to the manipulations of the experiments. The *second line* records the blood pressure, the cannula being in the carotid artery, the recording apparatus being the mercury manometer. The *third line* is the chronographic record (seconds). *a*, inflation begins; *b*, abdominal compression; *c*, respiratory efforts; *d*, inflation ends. Note the effect of inflation (rise of pressure followed by fall), effect of compression of abdomen repeated three times (rise but not above normal), onset of dyspnea, cessation of inflation with a slow rise of pressure above normal.

The electrocardiogram showed the following sequence of events: (1) a preliminary quickening;<sup>5</sup> (2) an initial slowing; (3) an accele-

<sup>5</sup> It should be remarked that the writers attribute no difference in meaning to these two terms (quickening and acceleration). The contrast is made merely for the sake of avoiding confusion.

ration; (4) a subsequent slowing which follows the cessation of the effort. Of these changes the subsequent slowing is the most constant; the initial slowing the least so. Thirteen experiments were performed on seven subjects.

1. *Preliminary quickening.* This fairly constant phenomenon is attributable to the deep inspiration which precedes the Valsalva experiment (cf. p. 482). It is the usual quickening which the heart normally exhibits as the accompaniment of inspiration. It differs only

TABLE 3  
*Changes in heart rate resulting from Valsalva experiment*

| SUBJECT              | PRELIMINARY QUICKENING | INITIAL SLOWING | ACCELERATION | SUBSEQUENT SLOWING |
|----------------------|------------------------|-----------------|--------------|--------------------|
| Dawson { no. 1.....  | Absent                 | Absent          | 80           | 170                |
| { no. 2.....         | Absent                 | Absent          | 50           | Absent             |
| Eyster.....          | 88                     | 117             | 88           | 125                |
| F. J. Hodges I.....  | 81                     | Absent          | 50           | 185                |
| P. C. Hodges II..... | 80                     | 133             | 73           | 140                |
| Kolls { I.....       | 80                     | 123             | Absent       | 143                |
| { II.....            | 87                     | Absent          | Absent       | 143                |
| { III.....           | 88                     | 114             | Absent       | 132 (1)            |
| Menninger { I.....   | 85                     | 117             | 56           | 156                |
| { II.....            | ?                      | 120             | 55           | 155                |
| { III.....           | 88                     | 123             | 66           | 142                |
| Nause { I.....       | 80                     | 116             | 64           | 133                |
| { II.....            | 70                     | Absent          | 56           | 122                |
| I Average.....       | 82.7                   | 120             | 63.8         | 145                |
| II Average.....      | 85                     | 112             | 72           | 142                |

Thirteen experiments on seven subjects have been recorded. In the *first* column are shown the names of the subjects and the designations of the different experiments performed upon them; in the *second*, the percentage of the preliminary quickening, the normal heart cycle being regarded as 100; in the *third to fifth* columns, the initial slowing, acceleration, and subsequent slowing are similarly dealt with. For (1) see page 505. No change of less than 10 per cent is regarded as exceeding the limits of normal variation and consequently such are designated "absent" in the table. The interrogation mark indicates that the electrocardiogram was imperfect so that the particular point designated could not be determined. The first average is that of experiments in which a change took place; the second that of all thirteen observations, "absent" being reckoned as 100. *Note relative constancy of subsequent slowing and variability of preliminary quickening, of initial slowing and of acceleration.*



in being more pronounced just as the inspiration itself is more pronounced than usual. This quickening was obvious in ten experiments out of twelve (table 3). The maximum reduction in the length of the cardiac cycle was to 70 per cent of its normal value.

2. *Initial slowing.* In eight cases out of thirteen there occurred either simultaneously with the beginning of the effort, or very soon after its beginning, a cardiac slowing which was sometimes very marked, the maximum increase in the cardiac cycle being 33 per cent (figs. 2 and 3). The duration of the slowing was short in every case, its greatest length being ten beats. The increase in rate which followed was often abrupt, giving a sharp angle to the charted curve (fig. 3). Since

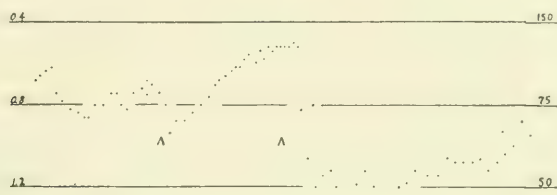


Fig. 2. The "typical" effect of the Valsalva experiment upon the heart rate. Each dot represents a single heart beat. Time is represented by distances along the abscissa; the numerals arranged in vertical column on the left represent the duration of the cardiac cycle in hundredths of a second; those in vertical column on the right represent the number of beats per minute corresponding to the figures in the first column. Of the wedges pointing upward that to the left indicates the beginning of effort, that to the right the end of the effort. Vertical broken line represents interval of 30 seconds between first and second part of figure. These explanations apply also to figures 3 to 5 and 8 to 15. Subject: Menninger (I). Note preliminary quickening, initial slowing, acceleration and subsequent slowing which has not passed off at the end of thirty-three seconds.

the period of slowing comes so soon after the beginning of the effort, one would *a priori* expect that it would coincide with the preliminary rise in systolic pressure. This expectation is borne out by the radial sphygmogram, for here the slowing of the pulse is often seen to accompany the reduction of the diastolic elevation (*vide infra*). Even when the effort is so severe as ultimately to suppress the pulse at the wrist during the fall of pressure, still during the part of the effort in which this slowing occurs, the pulse continues to be palpable.

3. *Acceleration.* Ten of the thirteen subjects showed a period of cardiac acceleration which varied much in amount, the maximum reduction in the length of the cycle being to 50 per cent. This phe-

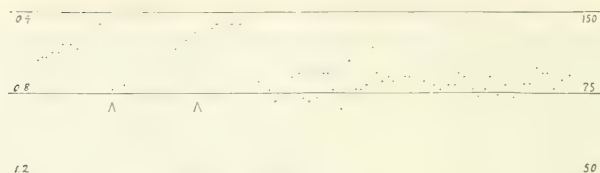


Fig. 3. "Typical" effect of Valsalva experiment upon heart rate. Subject: P. C. Hodges (II). For explanation of the symbols see legend of figure 2. Note preliminary quickening, enormous initial slowing, relatively small acceleration and moderate subsequent slowing, which has not disappeared at the end of forty-two seconds.

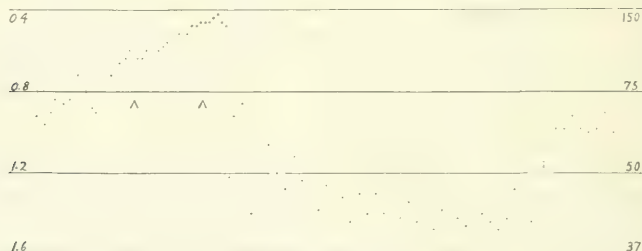


Fig. 4. Less "typical" effect of the Valsalva experiment upon the heart rate. Subject: F. J. Hodges (I). For explanation of the symbols see legend of figure 2. Note preliminary quickening, absence of initial slowing, presence of acceleration and of very pronounced subsequent slowing, which has returned to within nominal limits by the end of seventy-five seconds.

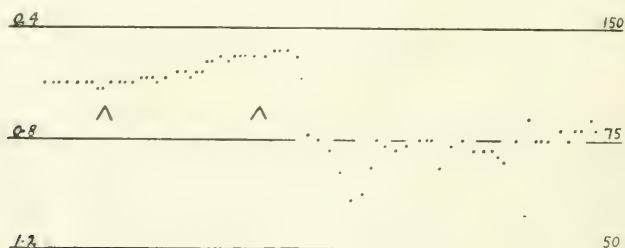


Fig. 5. Less "typical" effect of the Valsalva experiment upon the heart rate. Subject: Dawson (I). For explanation of the symbols, see legend of figure 2. Note the absence of preliminary quickening and of initial slowing, presence of slight acceleration, and marked subsequent slowing, which has not disappeared by the end of thirty seconds.

nomenon usually begins during the effort and continues from one to ten beats after the effort has ceased. It occurs, moreover, at about the time that the fall in blood pressures might be looked for, and might therefore be thought to be the accompaniment (or result) of the low pressure. Here again the sphygmogram confirms the a priori expectation in that the acceleration and the dicrotism are synchronous.

4. *Subsequent slowing.* In all but one experiment there occurred at from one to ten beats after the end of the effort, a very marked slowing of the pulse (figs. 2 to 5). Here the maximum lengthening of the cycle was to 185 per cent. The slowing was marked but usually not maximal at the outset. This final period was the longest of all, the slowing continuing often for forty or fifty beats and returning to normal sometimes with marked oscillations.

#### *Character of the changes in rate*

*Duration of Q-T complex.* If we assume that the duration of ventricular systole corresponds to the time interval between the Q- and T-waves of the electrocardiogram, then it is possible to determine the degree in which each of these phases of the cardiac cycle shares in the variations in duration of the complete cycle. A study of the electrocardiograms undertaken to elucidate this point shows that the variations are chiefly diastolic in character. The measurements in three typical experiments are presented in table 4.

*Duration of the atrio-ventricular interval.* Detailed study of the electrocardiogram was an after-thought. The string galvanometer was resorted to originally for the purpose of determining the heart rate after other methods had failed. The tracings were for the most part of little more value than was needed for the purpose of counting the heart beats, no efforts being made to cut out adventitious vibrations. In several experiments, however, the A-V interval could be made out.

This interval was occasionally altered. With a quickened heart beat there might be shortening of the A-V interval while with a slowing, the interval might be prolonged. Usually, however, the interval remained unchanged. The results relating to these points are embodied in the accompanying table (table 5). At one point (24 seconds) in the tracing obtained from F. J. Hodges (I) the P-wave could not be found. The heart was at this time beating very slowly and the picture was that of a nodal rhythm. The tracing is unfortunately not suffi-

ciently distinct to justify confidence, although suggestive enough to encourage further inquiry. In other tracings of hearts beating with equal slowness, the P-wave was clearly marked.

TABLE 4  
*Effect of Valsalva experiment on duration of Q - T complex*

| SUBJECT, ETC.                     | SECOND | DURATION OF |                    |                     |
|-----------------------------------|--------|-------------|--------------------|---------------------|
|                                   |        | Cycle       | Ventricle systolic | Ventricle diastolic |
| Dawson, no. 1, 6-20 seconds.....  | 49     | 1.00        | 0.24               | 0.76                |
|                                   | 65     | 0.76        | 0.26               | 0.50                |
|                                   | 5      | 0.60        | 0.28               | 0.32                |
|                                   | 23     | 0.58        | 0.26               | 0.32                |
| Dawson, no. 2, 6-19 seconds.....  | 23     | 0.50        | 0.23               | 0.27                |
|                                   | 4      | 0.82        | 0.26               | 0.56                |
|                                   | 29     | 0.82        | 0.26               | 0.56                |
|                                   | 16     | 0.50        | 0.24               | 0.26                |
| M€nninger, II, 16-34 seconds..... | 51     | 1.26        | 0.24               | 1.02                |
|                                   | 50     | 1.20        | 0.24               | 0.96                |
|                                   | 52     | 1.20        | 0.24               | 0.96                |
|                                   | 12     | 0.80        | 0.24               | 0.56                |
|                                   | 10     | 0.78        | 0.24               | 0.54                |
|                                   | 11     | 0.74        | 0.24               | 0.50                |
|                                   | 34     | 0.48        | 0.24               | 0.24                |
|                                   | 34     | 0.48        | 0.24               | 0.24                |
|                                   | 35     | 0.46        | 0.24               | 0.22                |

The *first* column shows the name of the subject to which is added a numeral indicating to which of the two or more experiments on this subject reference is made, also the numerical designation of the seconds at which the effort began and ended respectively, e.g., in Dawson no. 1., effort was begun during the 6th second and ended in the 20th after the beginning of the experiment. In the *second* column are the numerical designations of the seconds corresponding to the cycles analyzed in the succeeding columns. The *third, fourth and fifth* columns are the analyses of cardiac cycles of different lengths, taken from all parts of the electrocardiograms. *Note that duration of the cycle is mainly dependent on variations in diastole; while variations in systole are small and inconstant.*

*Variations in the Q-, R- and S-waves of the electrocardiogram.* The electrocardiograms were also examined to ascertain whether during the changes in rate there were any accompanying changes in the Q-R-S-complex. Nine tracings were found appropriate for this study (table 6). The changes observed were of three sorts, *a*, a general reduction



TABLE 5

*Effect of Valsalva experiment on atrio-ventricular interval*

| SUBJECT, ETC.                                      | SECONDS | CYCLE | A-V<br>INTERVAL |
|--|---------|-------|-----------------|
| Menninger I, 17-34 seconds, April 8, 1915.....     | 7       | 0.86  | 0.12            |
|  | 8       | 0.86  | 0.12            |
|  | 35      | 0.48  | 0.12            |
|  | 48      | 1.20  | 0.12            |
| P. C. Hodges I, 10-23 seconds.....                 | 8       | 0.60  | 0.12            |
|  | 31      | 0.84  | 0.12            |
| Menninger III, 23-69 seconds.....                  | 2       | 0.76  | 0.15            |
|  | 28      | 0.96  | 0.15            |
|  | 69      | 0.74  | 0.15            |
|  | 78      | 1.14  | 0.15            |
| Kolls II, 16-48 seconds, April 20, 1915.....       | 9       | 0.70  | 0.12            |
|  | 63      | 0.80  | 0.12            |
| Menninger II, 5-21 seconds, April 8, 1915.....     | 1       | 0.80  | 0.12            |
|  | 21      | 0.48  | 0.12            |
|  | 25      | 1.58  | 0.18            |
| Kolls III, 14-43 seconds, April 20, 1915.....      | 6       | 0.72  | 0.13            |
|  | 24      | 0.56  | 0.09            |
| F. J. Hodges I, 12-20 seconds, April 10, 1915..... | 6       | 0.72  | 0.12            |
|  | 7       | 0.80  | 0.12            |
|  | 8       | 0.88  | 0.12            |
|  | 23      | 0.48  | 0.08            |
|  | 24      | 1.22  | 0.00            |
|  | 37      | 1.32  | 0.12            |

Here the *first* column gives the name of the subject, the date of the experiment and the period of effort. The last is indicated by two hyphenated numbers, the first being the second during which the effort was begun, the second that during which the effort was ended. The *second* column gives the serial number of the second corresponding to the values which follow it on the same line; the *third* and *fourth*, the duration in seconds of the cardiac cycle and atrio-ventricular interval respectively. For example, in Menninger I thirty-five seconds after the beginning of the experiment (that is, during the second which followed the cessation of the effort) the duration of the cardiac cycle was 0.48 second, while that of the atrio-ventricular interval was 0.12. *Note that occasionally atrio-ventricular interval varies in same direction as duration of cycle.*

TABLE 6  
*Changes in the Q-, R- and S-waves due to the Valsalva experiment*

| NAME, ETC.                                     | PER<br>CENT | R- AND S-WAVES            |                    | S-WAVE                     |  |  | Q-WAVE             |
|--|-------------|---------------------------|--------------------|----------------------------|--|--|--------------------|
|  |             | Preliminary<br>quickening | Acceleration       |                            |  |  |                    |
| F. J. Hodges }<br>I, 13-20 }<br>seconds..... } | 50          | 10<br>0.72                | 20<br>0.48         | 21 23?<br>0.46 0.48        |  |  |                    |
| Kolls I, 18-32 }<br>seconds..... }             | 80          | 14<br>0.62                | 36<br>0.80         |                            |  |  |                    |
| Kolls II, 16- }<br>48 seconds.. }              | 87          | 14 16<br>0.56 0.60        | 26 43<br>0.60 0.68 |                            |  |  |                    |
| Menninger I, }<br>17-34 sec- }<br>onds..... }  | 56          | 14<br>0.68                | 34<br>0.48         | 21 34 36<br>0.68 0.48 0.48 |  |  |                    |
| Menninger }<br>II, 4-21 }<br>seconds..... }    | 55          | 3 7<br>0.78 0.86          |                    | 10 21 23<br>0.80 0.46 0.48 |  |  |                    |
| Dawson, no. }<br>1, 6-19 sec- }<br>onds..... } | 80          | 5 7<br>0.62 0.60          | 20 21<br>0.48 0.48 |                            |  |  | 7 22<br>0.60 0.58  |
| Dawson no. 2 }<br>5-19 sec- }<br>onds..... }   | 50          | 3 6<br>0.82 0.60          | 18 20<br>0.50 0.44 |                            |  |  | 12 23<br>0.60 0.58 |
| P. C. Hodges<br>II.....                        | 73          |                           |                    |                            |  |  |                    |
| Nause II.....                                  | 56          |                           |                    |                            |  |  |                    |

The *first* column shows name of subject, number of experiment on that subject and duration of effort, i.e., serial number of seconds during which effort began and ended; the *second* gives maximum reduction of duration of cardiac cycle, i.e., percentage of normal cycle to which the duration fell. The *third* and *fourth* columns are best explained by these examples: In Hodges I a decrease in the R- and S-waves began at 10th second (during preliminary quickening) and stopped at 20th second (during acceleration). Below figures 10 and 20 are figures 0.72, 0.48 indicating duration in seconds of corresponding cardiac cycles. A second example is that of Kolls II. Here there were two separate periods of reduction of waves in question, one during the preliminary quickening and one during the acceleration. The *fifth* column shows beginning of increase of S-wave, viz., 21, 21, 10, and disappearance of increase, viz., 23?, 36, 23. Where there are three consecutive figures as 21, 34, 36, then 21 denotes beginning of increase, 34 a sudden rise in the degree of increase and 36 disappearance of increase. Durations of corresponding cycles are placed below these figures. The *sixth* shows beginning and end of increase of Q-wave. *Note more constant presence of reduction of R-S waves, and relative infrequency of increase of Q-wave.*

in the size of the R- and S-waves; *b*, an increase in the Q-wave; *c*, an increase in the S-wave sometimes accompanied by a simultaneous decrease in the R-wave. No changes were observed when the heart became slower than normal but only when it quickened its pace. The general reduction in size occurred in seven experiments. In three of these the decrease began with the preliminary quickening and con-

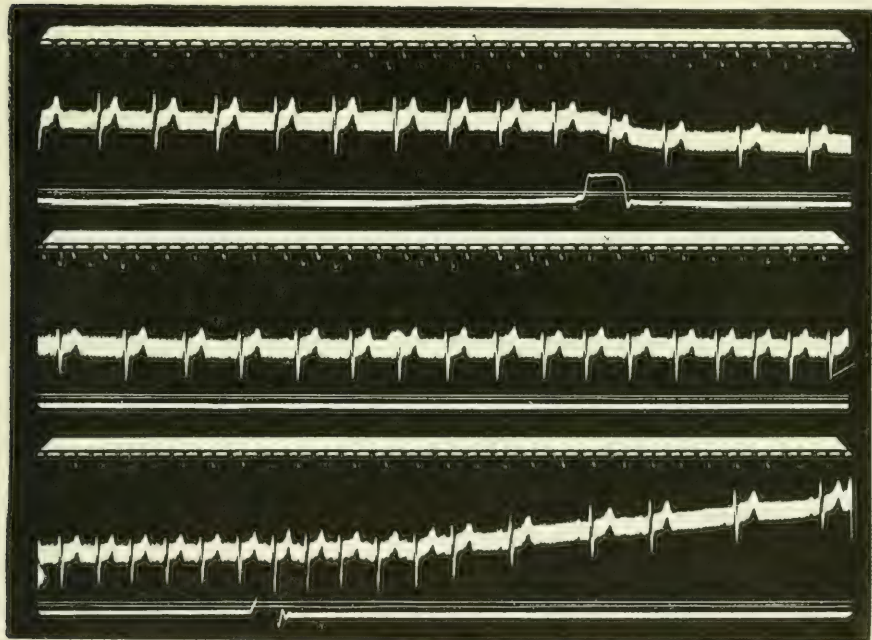


Fig. 6. Electrocardiogram taken during a Valsalva experiment. Subject: Menninger (II). Upper line is a chronographic record showing fifths of a second; middle line is the electrocardiogram; lower line, signal showing beginning and end of effort. Note preliminary quickening, initial slowing, acceleration with increase in S-wave, and subsequent slowing.

tinued all through the effort and for a few seconds after the effort was over. In three other experiments the decrease accompanied the preliminary quickening, then disappeared again during the acceleration. This change seems therefore not to be the result of the Valsalva experiment as such, but merely the effect of decreased vagal tonus. Finally in one case the change appeared during the preliminary quickening only.

The other two changes both imply an increase of the negativity of the apex as compared with the base of the ventricle. The increase in the Q-wave occurred in two experiments on the same subject. In one of them (Dawson no. 2) the phenomenon was very marked; in the other (Dawson no. 1) it was distinctly observable although less pronounced. The change began during the effort and persisted for a few seconds after the cessation of the latter. It should be noted that in neither of these experiments was there a preliminary quickening.

The increase in the S-wave sometimes began at the time of cessation of the effort (one experiment) and always lasted until the acceleration gave place to the subsequent slowing. It might, however, begin much earlier in the effort (two cases). It never accompanied the preliminary quickening and may therefore be inferred to result from the mechanical conditions of the Valsalva experiment itself.

In the present unsatisfactory state of our knowledge regarding the cause of the Q-, R- and S-waves and the significance of their variations, the writer feels that all he is justified in doing is to record the foregoing facts without attempting an interpretation. It is true that the tables might be turned and the facts in question might be utilized for the elucidation of the reverse problem, namely, the meaning of the Q-, R- and S-waves, but for dealing adequately with the latter further experimentation along these lines is desirable.

### *The sphygmogram*

Owing to some curious statements in the literature in regard to the significance of the sphygmogram obtainable during Valsalva's experiment (4), it seemed well to devote some attention to this matter. Of several sphygmograms obtained one is given as an example (fig. 7). Here it is seen that the normal pulse waves undergo a series of changes which may be described as follows: *a*, a fall of the base line; *b*, elevation of the base line, decrease in size of the beats and obscuration of dicrotic wave; *c*, fall of base line but not to normal, hyperdicrotism, and continued small size of pulse wave; *d*, return to normal by decrease in dicrotic wave, fall in base line and increase in size of pulse. One finds no difficulty in interpreting these changes: *a*, The fall in the base line is the result of the strong inspiration with which the Valsalva effort begins and which assists in emptying the radial venae comites. *b*, The rise in the base line is due to distention of the radial artery resulting from the rapid rise in arterial pressure and also to the filling of the



venae comites resulting from the rise in intrathoracic pressure which obstructs the venous outflow from the arm. *c*, As the arterial pressure falls rapidly, the base line sinks but does not reach normal since the veins are still engorged. *d*, With the return of normal pneumatic conditions within the thorax, the veins empty rapidly and the base line falls to normal. The hypodicrotism and the hyperdicrotism are evidences of high and low blood pressure respectively.

These explanations, as far as they go, seem to the writers to accord well with known facts and to render the efforts of some investigators not a little superfluous.

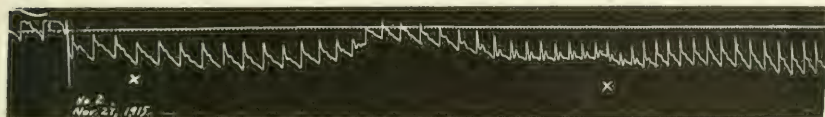


Fig. 7. Radial sphygmogram during Valsalva experiment. Chronograph records fifths of a second. *x* indicates approximately beginning and end of experiment including preliminary slow and deep inspiration. Note variations in base line (an index of engorgement of radial vessels), and in height of dicrotic elevation (an index of arterial pressure).

#### LIFTING

It has been the view usually accepted that lifting and the Valsalva experiment are essentially alike insofar as the disturbances of the circulation are concerned. This statement is in the main correct. The blood pressures and pulse rate usually do show a picture which corresponds to that seen in the Valsalva experiment although often these changes are less pronounced. As in the case of the Valsalva experiment, there are two series of variations to be considered, the blood pressure changes and the variations in the pulse rate. We shall consider these in turn.

#### *Blood pressure (systolic)*

The blood pressure changes will be rather summarily dismissed, otherwise, because of the essential resemblance between the effect of lifting and that of the Valsalva maneuver, we would only be engaging in vain repetitions. Three experiments were performed and showed the *primary rise* and the *fall* but no second rise until after the cessation of the effort.

It is, however, highly desirable that these studies should be carried further. Enough material has not been gathered to enable the writer to study individual variations.

### *Pulse rate*

The pulse rate on the other hand received adequate attention. Its behavior also resembles in the main that observed in the Valsalva experiment. There are, however, a few exceptions as will be seen presently.

The method employed was as follows: The subject stood upon a strong square piece of wood (about 80 x 80 cm.) to the center of which was fastened by a hook a registering spring dynamometer. The subject stood over the hook and passed around the body and over one shoulder a sling which was secured to the free pole of the dynamometer. The sling was then shortened until the subject stood with the knees slightly bent. At the word of command the legs were extended and the subject made a vigorous attempt to lift the square piece of wood upon which he stood. The amount of effort was registered by the dynamometer. The subject ceased his effort with a loud gasp which was recorded by the operator who had also marked upon the electrocardiographic tracing the moment at which the command to lift had been given. During the whole of this procedure electrocardiograms were being taken from the two forearms.

The alterations of the pulse rate are presented best by means of the accompanying table (no. 7) and charts (8 to 15). The former contains most of the data embodied in the discussion while the latter are shown chiefly for the purpose of illustration.

On perusing table 7 one becomes aware of the following facts: *a*, In general the changes in heart rate are similar to those produced by the Valsalva experiment, but are quantitatively less. *b*, The preliminary quickening is a very inconstant phenomenon. This accords with the supposition already advanced that the preliminary quickening is due to an accentuation of inspiration for it often occurs that one does not precede an effort at lifting by taking a deep breath. *c*, The initial slowing occurred in numbers 1 and 4, but was absent from numbers 6 and 12. This may indicate that the phenomenon in question is at least partly dependent upon the severity of the lift for the dynamometer record was lower in numbers 6 and 12 than in numbers 1 and 4. *d*, The subsequent slowing is one of the most constant features; its

appearance may however be delayed for as much as one minute (nos. 5 and 10). It is not always the last sign to disappear when the effort is reduced for although this would seem to be the case sometimes (no. 12) it is not always so (no. 15). *e*, The acceleration is also a pretty constant feature. It may extend throughout most of the period of effort (nos. 5 and 7). On increasing the effort, it may linger after the secondary slowing has disappeared or on decreasing disappear before it (nos. 15, 12). *f*, The erratic records (nos. 7, 13 and 14) require special con-

TABLE 7  
*Changes in heart rate due to lifting*

| NUMBER           | SUBJECT, ETC.      | PRELIMINARY QUICKENING | INITIAL SLOWING | ACCELERATION | SUBSEQUENT SLOWING |
|------------------|--------------------|------------------------|-----------------|--------------|--------------------|
| 1                | Hodges I.....      | ?                      | 126             | Absent       | 166                |
| 2                | Jolivette.....     | 89                     | Absent          | Absent       | 139                |
| 3                | H'Doubler II.....  | 88                     | Absent          | 82           | 123                |
| 4                | Pressentin I*..... | Absent                 | 120             | 89           | 120                |
| 5                | Bodman.....        | Absent                 | Absent          | 80           | 116 (1)            |
| 6                | Hodges II.....     | 85                     | Absent          | 66           | 134                |
| 7                | Dawson I.....      | Absent                 | Absent          | 86 (2)       | 140                |
| 8                | Schlatter*.....    | Absent                 | Absent          | 75           | 159                |
| 9                | Kriskey.....       | Absent                 | Absent          | 70           | Absent             |
| 10               | Dawson II*.....    | Absent                 | Absent          | 79           | 152 (3)            |
| 11               | Morris.....        | 87                     | Absent          | 78           | 177                |
| 12               | Pressentin II..... | Absent                 | Absent          | Absent       | 113                |
| 13               | H'Doubler I.....   | ?                      | Absent          | 97, 114 (4)  | 125                |
| 14               | Kelley*.....       | ?                      | Absent          | 121, 146 (5) | 128                |
| 15               | Dawson 18.....     | Absent                 | Absent          | 74           | Absent             |
| 16               | Dawson 19.....     | Absent                 | Absent          | 60           | 114                |
| I Average .....  |                    | 87                     | 123             | 76           | 136                |
| II Average ..... |                    | 96                     | 102             | 81           | 131                |

The *first* column gives reference numbers which are used in discussion in text. For remaining columns see legend of table 3. No change of less than 10 per cent is regarded as exceeding the limits of normal variation and consequently such changes are designated "absent" in table. Interrogation point and averages as in table 3. (1) Signifies maximum change reached late (60 seconds); (2) interrupted by a slowing (see page 505); (3) coming on late; (4) omitted from both averages; here a slight acceleration occurred (97 per cent) but the last and longest part of effort was accompanied by slowing (114 per cent maximum); (5) omitted from both averages; here usual acceleration was replaced by slowing, shortest cycle during effort was 121 per cent, the longest 146 per cent. In numbers 1, 4 and 15 effort was much more severe than in numbers 6, 12 and 16 respectively. \*Shown in figures. *Note that changes although essentially similar to those due to Valsalva experiment are less in amount.*

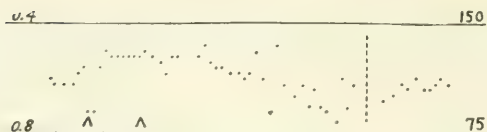


Figure 8. More "typical" effect upon the heart rate of lifting. Subject: Pressentin (I). For explanation of symbols see legend of figure 2. *Note the initial slowing, acceleration and subsequent slowing.*

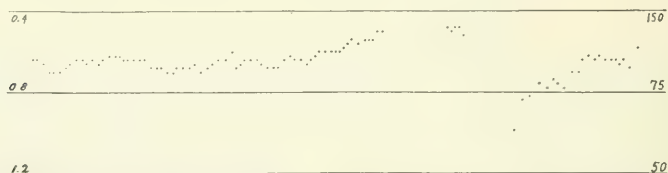


Fig. 9. Less "typical" effect of lifting upon the heart rate. Subject: Schlatter. For explanation of the symbols see legend of figure 2. *Note the absence of the preliminary quickening, and the initial slowing. The acceleration and subsequent slowing are still pronounced.*

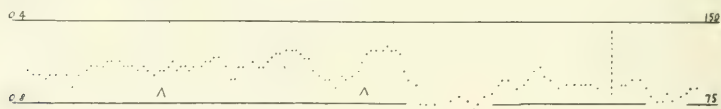


Fig. 10. Less "typical" effect of lifting upon the heart rate. Subject: Dawson (I). For explanation of the symbols see legend of figure 2. *Note the absence of the preliminary quickening and the initial slowing; the presence of two periods of acceleration separated by a slight slowing, and of the subsequent slowing.*

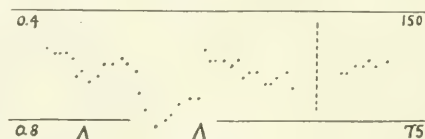


Fig. 11. "Atypical" effect of lifting upon the heart rate. Subject: Kelley. For explanation of symbols see legend of figure 2. It is exceptionally unfortunate that in this case the gap in the record (the half-minute pause) occurs where it does. It is possible that during this interval the usual secondary slowing may have taken place. *Note the presence of the initial slowing, of a return to normal following the cessation of the lift.*



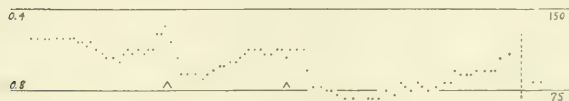


Fig. 12. First of three figures showing the effect upon the heart rate of prolonging the Valsalva experiment. Subject: Kolls (I). For explanation of the symbols see legend of figure 2. This short effort is presented for the sake of comparison with figures 13 and 14. All three were obtained from the same subject. *Note typical picture consisting of preliminary quickening, primary slowing, acceleration and subsequent slowing.*

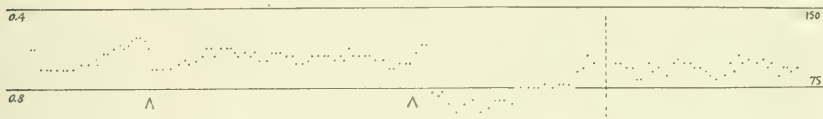


Fig. 13. Second of three figures showing the effect upon the heart rate of prolonging the Valsalva experiment. Subject: Kolls (II). For explanation of the symbols see legend of figure 2. This effort is longer (32 seconds) than that of the preceding figures (14 seconds). *Note preliminary acceleration, very slight primary slowing (which amounts merely to a return to normal), moderate secondary acceleration, subsequent slowing.*

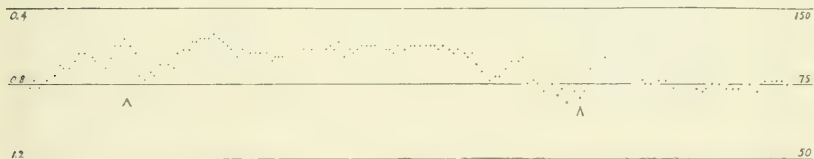


Fig. 14. Third of three figures showing the effect upon the heart rate of prolonging the Valsalva experiment. Subject: Kolls (III). For explanation of the symbols see legend of figure 2. This long effort lasted sixty seconds. *Note the preliminary acceleration, the primary slowing, the acceleration, the subsequent slowing. There is also a period of slowing before the official end of the effort.*

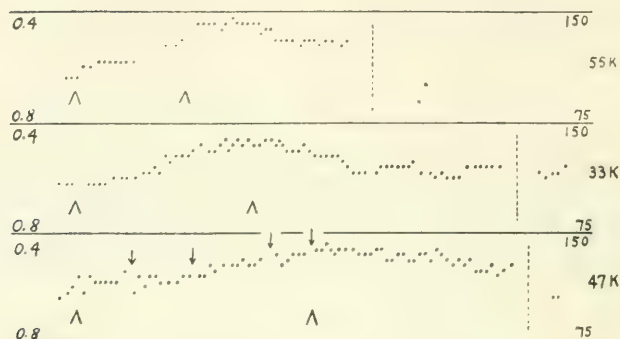


Fig. 15. The effect of lifting upon the heart rate as modified by the severity of the effort and by gasping. Subject: Dawson. For explanation of the symbols see legend of figure 2. The three curves are constructed on the same scale. The arrows pointing downward toward the third curve indicate the moments at which the subject gasped while making this record. The weights lifted which correspond to the three curves are, when arranged in order from above downward, 55, 35 and 47 kilos. Note that the last curve shows no greater deviation from the normal than the second curve excepting the fact that the acceleration took longer to pass away. This is in spite of the greater effort as compared with the second.

TABLE 8

*Effect of lifting on duration of Q-T' complex*

| SUBJECT, ETC.                    | SECOND | DURATION OF |                      |                       |
|----------------------------------|--------|-------------|----------------------|-----------------------|
|                                  |        | Cycle       | Ventricular systolic | Ventricular diastolic |
| Dawson II, 15-36 seconds.....    | 48     | 1.04        | 0.22                 | 0.46                  |
|                                  | 39     | 0.80        | 0.22                 | 0.24                  |
|                                  | 13     | 0.68        | 0.22                 | 0.46                  |
|                                  | 26     | 0.48        | 0.24                 | 0.24                  |
| Pressentin I, 3-10 seconds.....  | 22     | 0.68        | 0.24                 | 0.44                  |
|                                  | 1      | 0.60        | 0.24                 | 0.36                  |
|                                  | 12     | 0.52        | 0.22                 | 0.30                  |
| Pressentin II, 3-30 seconds..... | 36     | 0.64        | 0.24                 | 0.40                  |
|                                  | 2      | 0.60        | 0.22                 | 0.38                  |
|                                  | 27     | 0.56        | 0.22                 | 0.34                  |

For explanation see legend of table 4. Note similarity of this table to corresponding table for Valsalva experiment (no. 4).

sideration: 1, The record no. 7 is easily interpreted by supposing that a fluctuation of the attention caused this subject to relax his efforts and then to reinforce them just before the cessation of the pulling. 2, The record no. 13 might be explained in the same way, but for the fact that we would then expect the slowing to continue after the cessation of the effort until the final return to normal. If this objection be valid we are left without an explanation of this record. 3, The record no. 14 is characterized by a great slowing during the effort. Here again one might urge that the subject ceased her efforts before she was aware of it, and that the actual termination of the effort does not coincide with the intentional termination. But the same objection that has just been advanced in the case of no. 13 applies here with redoubled force for the unusual slowing does not persist after the effort has come to its intentional conclusion but is actually replaced by a marked acceleration. The justifiable supposition is that the intentional termination of the effort is the actual termination of the effort, but if so the unusual slowing remains without an explanation (cf. pp. 505 and 485).

*Duration of the Q-T complex.* Three electrocardiograms were found suitable for this study and the results were similar to those occurring in the case of the Valsalva experiment (table 8).

#### *Character of the changes in rate*

*Duration of the atrio-ventricular interval.* What has already been said in regard to the unsatisfactory character of the electrocardiograms in the case of the Valsalva experiment applies with still greater force in the case of the lifting experiments. We shall however consider such results as have been obtained. Of seventeen experiments only one showed a P-wave of adequate distinctness, the rest being spoiled by vibrations due to adventitious alternating currents. This single record (Dawson I) showed no change in the atrio-ventricular interval although the cycle was shortened from 0.64 to 0.44 seconds. These cycles occurred in the second and seventeenth seconds respectively, the period of effort being from the third to the twelfth seconds.

*Variations in the Q-, R- and S-waves of the electrocardiogram.* As in the case of the Valsalva experiments the electrocardiograms were also examined to ascertain whether during the changes in rate there occurred any modifications of the Q-, R- and S-waves. Ten of the seventeen experiments were found satisfactory for this study and the three changes observed in the case of the Valsalva experiment were noticed, and shall now be considered in order.

1. General decrease in the size of the R- and S-waves. Five of the experiments were negative while four gave results similar to those obtained in the Valsalva experiments (table 6). The remaining experiment (Kelley) differed entirely from the rest. Here the maximum acceleration was to 94 per cent but the record showed that during

TABLE 9  
*Changes in the R- and S-waves due to lifting*

| NAME, ETC.                      | PER CENT    | R- AND S-WAVES         |           |                |            |
|---------------------------------|-------------|------------------------|-----------|----------------|------------|
|                                 |             | Preliminary quickening |           | Acceleration   |            |
| Kelley, 4-14 seconds..... {     | 94<br>(144) | (3)<br>(0.36)          |           | (14)<br>(0.68) |            |
| Jolivette..... {                | 92          | 5<br>0.56              | 7<br>0.54 | 10<br>0.60     | 16<br>0.66 |
| Pressentin II.....              | 90          | No change              |           | No change      |            |
| H. Doubler II..... {            | 84          | 3<br>0.60              |           | 22<br>0.60     |            |
| Pressentin I.....               | 80          | No change              |           | No change      |            |
| Bodman.....                     | 79          | No change              |           | No change      |            |
| Dawson 18.....                  | 74          | No change              |           | No change      |            |
| Kriskey..... {                  | 70          | 6<br>0.56              |           | 19<br>0.46     |            |
| Dawson II, 15-36 seconds..... { | 67          | 15<br>0.64             |           | 36<br>0.60     |            |
| Dawson 19.....                  | 65          | No change              |           | No change      |            |

For explanation see table 6. The figures in parentheses are the discordant results (Kelley) referred to in text. *Note similarity of this table to corresponding table for Valsalva experiment (no. 6).*

the greater part of the lift the duration of the cycles was increased, attaining a maximum of 144 per cent. Along with this increase in duration of cycle there was a marked increase in the R-Q complex lasting from the third to the fourteenth second. No explanation is offered at the present time for this phenomenon.



2. Increase in the S-wave was not found at all, and

3. Increase in the Q-wave was found in only one instance at the period of maximum acceleration when the duration of the cycle had been reduced from 0.58 to 0.46 seconds.

#### CERTAIN MODIFICATIONS OF LIFTING AND THE VALSALVA EXPERIMENT

The effect upon the heart rate of prolonging the Valsalva experiment was studied in a single case. (Figs. 11, 12 and 13.) Here the usual four phases appeared in the first, the shortest, effort. On lengthening the period of effort there was no conspicuous change in the character of the results. But when the period was still further lengthened there appeared a marked slowing before the recorded end of the effort. On this record is marked the only *intentional* termination of the effort but if the attention or strength of the subject should flag during the experiment and the effort therefore be relaxed, a considerable amelioration of the pressure conditions within the thorax would result without the subject becoming aware of it. This state of things might give evidence of its existence through a premature slowing. The latter would disappear again when the effort was reinforced as it would be prior to the intentional abandonment of the effort which brings the experiment to its official termination.

Support is given to this explanation by the somewhat similar results shown in figure 10. Here the writer (Dawson) was the subject and was watchful of his mental states during the course of the experiment (lifting). A fluctuation in the degree of effort was synchronous with a premature slowing which was succeeded by an acceleration followed in turn by a second slowing, the one which we have been designating the subsequent slowing.

It has been seen that the pulse variations are of two sorts, the preliminary slowing which coincides with a respiratory movement, and the other three phases which are synchronous with changes in pressure. The initial slowing accompanies the primary rise in pressure; the acceleration, the fall in pressure and the subsequent slowing, and the return of the blood pressure to or above normal. It is a suggestive thought although perhaps not a justifiable assumption at the present time to regard the changes in heart rate as indices of blood pressures. In such a thought or assumption it is implied that in Kolls III there was a *secondary rise* in systolic pressure comparable to that described by Bruck (1).

*The effect upon the systolic blood pressure and pulse rate of gasping during the Valsalva experiment and during lifting* was studied in several instances. This seemed desirable because of the fact that certain forms of physical effort are characterized by periods of strain interrupted by gasps. All very prolonged strains are so interrupted. Observations upon the blood pressure changes were confined to the Valsalva experiments while those dealing with the heart rate were concerned with lifting only.

In the former study the method employed was the following: The pressure in the cuff of Erlanger's sphygmomanometer was raised to a high level and allowed to fall slowly during the performance of a Valsalva strain interrupted by gasps. At every 5 mm. of fall the observer marked with a key upon the drum. When the record was completed the pulse curves were examined to locate the first appearance of pulse tracings showing one of the systolic characters, namely, the separation of the catacrotic from the anacrotic limb. The pressure (shown by the manometer) which was synchronous with this form of pulse wave was taken to be the systolic pressure at that moment.

In three experiments in which the manometer pressure fell from 180 mm. the systolic pressure was found to be 140, 130 and 130 mm. respectively, during gasps. The normal systolic pressure of the subject observed was 118 mm.

In studying the heart rate the usual electrocardiographic method was employed. The results obtained are shown in fig. 15. Three experiments were performed upon the same subject (Dawson). In the first 55 kilos were lifted, in the second 35, and in the third 47. The duration of the lift was progressively longer in the order given (10, 16 and 21 seconds respectively) and during the third effort the subject gasped repeatedly. As shown in the figure the changes in rate were greatest in the first experiment while those in the other two were about equal, the somewhat greater acceleration in the second being balanced by the greater subsequent slowing in the third. Thus the changes produced in the third experiment (with gasping) were no greater than those in the second in spite of the fact that in the third experiment the duration of the lift was 34 per cent longer and the weight lifted 37 per cent heavier.

## LABOR PAINS

As already stated in the introduction, parturition resembles considerably the Valsalva experiment in its mechanics. There is here the same closure of the glottis and forced expiratory effort, and we may infer that the cardio-vascular reaction is similar in the two phenomena.

A chance observation upon a rabbit which was unexpectedly found to be pregnant during the course of an experiment seems worthy of being recorded. In one of a series of experiments on the blocking of nerve impulses through the application of heat, the sciatic of this rabbit which was under the influence of chloral, urethane and ether was stimulated with a moderate tetanic current. This stimulus was



Fig. 16. Changes in respiration and mean blood pressure during labor. Subject: rabbit under chloral, urethane and ether. *Upper line* records respirations by means of tambour connected with trachea cannula; *middle line*, mean blood pressure recorded by means of cannula in carotid artery; *lower line*, seconds and zero pressure. Mean pressure before spasm 114 to 122 mm., at height of rise 128 mm., at depth of fall 110 mm. Hg. The record shows no change in rate of heart rate nor of respiratory movements. *Note the decrease and cessation of respiration, a rise followed by a fall in mean pressure.*

sufficient to start a series of labor pains which resulted in the abortion of several nearly-mature fetuses. As the carotid of the rabbit was at the time connected with a mercury manometer, a record of the mean blood pressure was obtained during these labor pains. The respiration was also being recorded by means of a side tube from the trachea cannula.

On examining this record (fig. 16) one observes a uniform movement of the respiratory recorder and of the mean blood pressure. The latter shows waves of the first (cardiac) and of the third order (vasomotor) (5). The waves of the second order (respiratory) are absent. From the normal level the mean pressure rises slowly for the

duration of about three of the large waves. It now rises sharply, next falls more slowly to a level which is subnormal and from which it returns to normal in the duration of about three of the large waves (sixty seconds).

These pressure phenomena are not dissimilar to those obtained in the case of the Valsalva experiment but since the closure of the glottis has been rendered ineffective by tracheotomy, the changes in question must result from a totally different set of causes. It is suggested that the cause in this case is the increase in peripheral resistance due to the contraction of the uterus. The writers have frequently observed a well marked and similar pressure change in the rabbit on stimulation of the peripheral end of the sciatic where the only area of constriction was the lower leg and foot and it seems not unnatural to suppose that these changes in pressure may readily result from the constriction and relaxation of so large and vascular an organ as the uterus at term.

During the sharp rise in mean pressure the respirations show a short increase, a diminution for several movements, a cessation for a couple of seconds and as the pressure falls a rapid return. The subsequent movements are at first supernormal but normality is reached in about thirty-five seconds. The respiratory pause seems to have been in inspiration for the last weak movement was inspiratory (a down stroke) and the first of the returning movements expiratory (an upstroke).

Under the conditions which existed in this animal, one would have expected the respirations to show first a deep inspiration followed at once by the strong although mechanically ineffective expiration. The phenomena observed, namely, gradual suppression and gradual return of the respiratory movements, are puzzling and the writers do not hazard a conjecture as to their significance. Suffice it then to emphasize that in constructing a picture of the blood pressure changes in parturition one must include changes which are independent of the closure of the glottis.

#### SUMMARY OF RESULTS

The following summary is submitted without further discussion. Credit to previous investigators together with the comment and criticism of one of us (D) will be reserved for the historical résumé already referred to (footnote 3, p. 482).

I. In the Valsalva experiment the following phenomena were observed:



1. The systolic pressure rose rapidly, primary rise (McCurdy). This was followed by an extensive fall (Valsalva and Weber).

2. In place of the extensive secondary rise described by Bruck (to 180–200 mm.) a rise to not more than 140 mm. was observed, i.e., a height readily accounted for by the mild degree of asphyxia which occurred in these experiments.

3. When in the anesthetized dog the inflation of the lungs had reduced the mean blood pressure by 92 mm., neither violent abdominal compression nor severe dyspnea raised this pressure above normal again. On relieving the inflation the mean pressure rose somewhat above normal.

4. On cessation of the Valsalva effort there was a moderate rise of the systolic pressure.

5. The pulse rate showed a preliminary quickening which accompanied the inspiratory movement (cardiac cycle was sometimes reduced by 30 per cent), an initial slowing (cardiac cycle was sometimes increased by 33 per cent), an acceleration (cardiac cycle sometimes decreased by 50 per cent). The last usually began during the effort and continued for several beats after the effort had ceased, often not reaching its maximum until that time.

6. Subsequent to the effort there was a slowing (cardiac cycle sometimes lengthened by 85 per cent) which might continue for 40 to 50 beats.

7. As the time which elapsed between the Q- and T-waves (electrocardiographic) showed but little variation, the changes in the cardiac cycle may be regarded as chiefly diastolic.

8. The atrio-ventricular interval was usually unchanged but might be shortened with the shortening of the cardiac cycle.

9. The R- and S- waves might be reduced during the preliminary quickening or the acceleration or both. The acceleration might be accompanied by an accentuation of the Q-wave, or by an increase of the S- with a simultaneous decrease of the R-wave.

10. The changes in the sphygmogram were readily attributable to the changes in venous and arterial pressure.

II. Lifting was characterized by the following phenomena:

11. The changes in systolic blood pressure were essentially similar to those which occurred in the Valsalva experiment.

12. The changes in pulse rate were often essentially similar to those occurring in the Valsalva experiment. They were, however, less regularly present and might be less in amount. In order of the decreas-

ing frequency of their occurrence they were *a*, subsequent slowing and acceleration; *b*, initial slowing; *c*, preliminary quickening.

13. The shortening of the cardiac cycle was chiefly diastolic. The R- and S-waves might show a simultaneous decrease. The Q-wave was sometimes accentuated. No increase in the S-wave was noted (with the exception mentioned on page 504, viz., no. 14).

III. When the Valsalva or lift was modified, the usual picture was somewhat changed as follows:

14. The effect upon the heart rate of greatly prolonging the Valsalva experiment was to decrease the intensity of the effort and with this the extent of the changes in the heart rate.

15. When the Valsalva experiment experienced a series of *interruptions* consisting of a single gasp each, the systolic blood pressure rose with each gasp 10 to 20 mm. above normal.

16. When a lifting experiment experienced similar interruptions, the changes of heart rate were less in extent than would otherwise occur even when the weight lifted was greater in amount.

IV. Observations made during parturition:

17. During a labor pain in the anesthetized and tracheotomized rabbit, the mean blood pressure experienced changes (rise followed by fall) which are attributable to the changes in peripheral resistance due to uterine contraction and subsequent relaxation.

18. The respiration also changed decreasing in amplitude to a standstill in the inspiratory phase and then gradually returning to normal.

The writers have much pleasure in acknowledging the assistance of those students of medicine and of physical education who served as subjects in this research. To the names already mentioned in the text should be added those of Misses Glassow and McFadden.

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## SOME ASPECTS OF THE NEUROMUSCULAR RESPIRATORY MECHANISM IN CHELONIANS

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In 1795 Robert Towson, at Göttingen, first showed that tortoises do not swallow air, as had previously been believed, but that by the contraction of certain muscles, the lung is compressed and expels air, "then, ceasing to contract, the other muscle contracts and draws the former, (e.g., the lungs), within; thus a vacuum is formed into which the air rushes, as in the respiration of animals with a thorax" (1).

In 1863 Mitchell and Morehouse (2) described the normal respiration of the turtle as being due to the alternate contractions of certain antagonistic groups of muscles. "Inspiration is effected by the contraction of the flank muscles, which in appearance strongly resemble the diaphragms of superior animals. Expiration is effected by muscles which lie within the breast-box and consist of anterior and posterior bellies connected by a strong tendon continuous across the mid-line and common to both sides of the animal. These muscles act together and compress the viscera against the lungs." According to these authors the lung does not take an active part in the respiratory movements, but is compressed by the contraction of the expiratory muscles, while its passive expansion, due to the contraction of another set of muscles, causes more air to rush in and fill the lung cavity as in mammalian respiration. The same authors found that the neural apparatus of the respiratory mechanism consists essentially of the vagus supplying the larynx and lungs, of the spinal nerves distributed to the respiratory muscles of the trunk, and of the medulla oblongata through which the synchronous movements of the glottis and flanks are controlled. The functional activity of the vagus in connection with the lungs is not emphasized, however, in view of the passive rôle played by the lungs in normal respiration.

In 1878 Paul Bert made further observations, confirming the fact that respiration is carried on by contraction and dilatation of the

thoracic-abdominal cage. He also made the important observation that the lungs themselves can be made to contract by direct electrical stimulation (3).

François-Frank, in 1906, made a comprehensive and detailed study of the muscular mechanism of respiration in the turtle, with an analysis of the normal respiratory curves. He studied the lungs and their innervation by the vagus. He also observed spontaneous contractions of the lung tissue so long as the medulla was intact (4).

In 1908 Prévost and Saloz (5) called attention to the fact that stimulation of the vagus produces constriction of the bronchioles in the turtle, and in 1918 Jackson and Pelz demonstrated that while stimulation of the vagi produces constriction of the lungs, stimulation of the sympathetic chain with a very weak tetanizing current produces dilatation (6).

In this paper the experimental work under discussion is twofold, dealing with the activity of the lungs themselves under certain conditions, and the effects of experimental lesions of the cerebrum, optic lobes and medulla upon the respiratory muscles which are effective in maintaining normal respiration.

The activity of the lungs is first considered. Turtles were lightly etherized and the cranial cavity was opened. The cerebral hemispheres were removed, and the technique followed was that devised by Jackson in which, after removing the plastron, the fore and hind limbs were removed as well as all the viscera except the lungs and heart. The latter was connected with a recording lever. The vagi were freed for some distance in the neck, and ligatures were placed loosely around them. A small glass cannula was inserted in the trachea, by means of which, after being half inflated, the lungs were connected with a very sensitive recording tambour, which indicated very small changes in volume. A small pair of electrodes, insulated except at the very point, was then fastened firmly, by means of adhesive tape, into the cranial cavity on the optic lobes, which were then stimulated with a tetanizing current. In order to check up results as being due to conduction by nerve fibers and not to escape of current in the cranial cavity, whenever practicable before the close of an experiment the optic lobes were divided from the brain stem and again stimulated with an induction current of the same intensity as before.

Following is the protocol of a typical experiment.



*May 20, 1919*

Turtle prepared according to Jackson's technique.

Optic lobes stimulated for 20 seconds with medium induction current. Good contraction of the lungs. Repeated.

Right vagus stimulated with medium induction current for 20 seconds. Contraction of the lungs with inhibition of the heart. Repeated.

Right vagus divided.

Left bronchus occluded with artery clip, optic lobes stimulated. No contraction of right lung.

Right bronchus occluded, left released. Optic lobes stimulated. Contraction of left lung. Repeated.

Optic lobes divided from brain stem, then stimulated. No contraction of left lung.

With a moderate tetanizing current, upon stimulation of the optic lobes or the medulla, I have uniformly obtained a rise in the point of the recording lever, similar to that obtained by stimulation of the peripheral end of the vagus, except that there is no such inhibition of the heart as occurs in the latter case. As figure 1 shows, the heart beat is uninterrupted, although the contraction of the lungs is identical with that which accompanies inhibition of the heart on stimulation of the vagus. This contraction is long and slow with a long latent period—a very different type of response from a normal respiration, in which there is a sudden sharp contraction of the muscles of the thoracic-abdominal cage with an expulsion of about two or three times the volume of air expired in a simple contraction of the lungs. Inhibition of the heart occurs only when a current of greater intensity than that necessary to produce contraction of the lungs is employed. Neither stimulation of the optic lobes nor of the medulla oblongata has ever produced dilatation of the lungs.

The single contraction upon stimulation of the optic lobes or medulla with the tetanizing current is a typical smooth muscle contraction, such as one obtains from the urinary bladder of the cat when the hypogastric nerves are stimulated. Such a contraction as the result of stimulation may be shown with one lung by placing a light artery clip upon the main bronchus of the other side and thus occluding the opposite lung. If this is done, and the optic lobes are stimulated, a normal response is elicited from the side under observation. But if the vagus to this lung be now cut, and stimulation again be done, there is no contraction. If the bronchus on the side on which the vagus has been cut is now occluded with the artery clip and the other side is freed, on stimulation of the optic lobes there is a normal response from the normal side.

Such behavior apparently indicates that in the turtle the fibers of the vagus which pass to the lungs have anatomically different nuclei of origin from the fibers which go to the heart.

During a number of experiments, I observed in detail a phenomenon of which François-Frank speaks. This is the spontaneous rhythmic



Fig. 1. Upper tracing, response of right lung (vagus intact) to stimulation of the optic lobes. Lower tracing, heart beat.

contraction of the lungs of the turtle similar to that evoked by stimulation of the brain stem. Unlike the spontaneous contractions of smooth muscle, which take place independently of a centrifugal mechanism, the medulla and vagi of the turtle must be intact,—a demonstration of the fact that a central origin and efferent fibers are necessary to produce the contractions.

We now pass to a consideration of the neuromuscular mechanism which is effective in maintaining normal respiration. In the turtle the muscular mechanism, as earlier authors have shown, is the thoracic-abdominal cage, in which occur alternate contractions and relaxations of antagonistic groups of muscles. The innervation of these muscles is similar to that of other vertebrate forms, namely, by the afferent and efferent nerves which enter and emerge from the spinal cord at

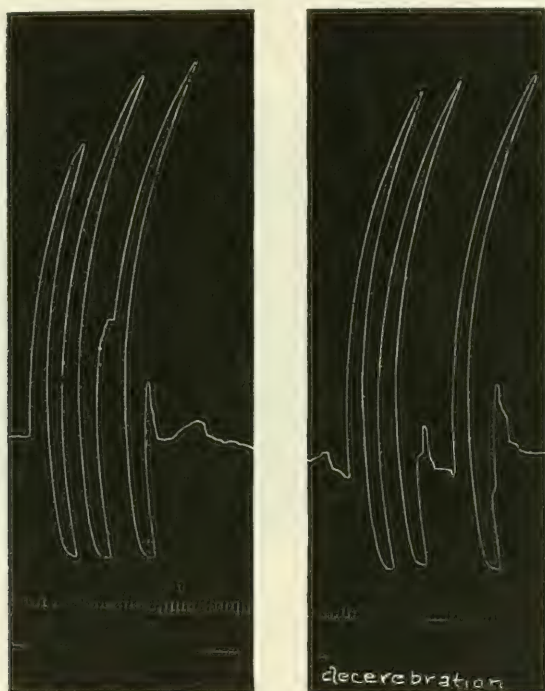


Fig. 2. Tracing 1, Normal respiration. Tracing 2, Respiration after removal of the cerebral hemispheres.

corresponding levels. The respiratory impulse is initiated in the respiratory center of the medulla oblongata and passes by descending tracts in the spinal cord to the motor nerves of the muscles which effect respiration, just as in mammalia.

Previous authors have agreed that so long as the medulla is intact, respiration continues, at slightly varying intervals of time, but if the medulla be separated from the spinal cord, respiration is abolished permanently.

In this study I have endeavored to investigate further,

a. The effect upon respiration of lesions above the medulla.

b. The effect upon respiration of certain lesions of the medulla oblongata.

In these experiments, normal turtles were lightly etherized and a cannula was inserted in the trachea. This was connected by means of double valves (one for intake and one for outlet) with a Verdin recording tambour. Tracings of normal respiratory curves were obtained. The cerebral hemispheres of the animals were then removed and respiratory tracings were again taken (fig. 2).

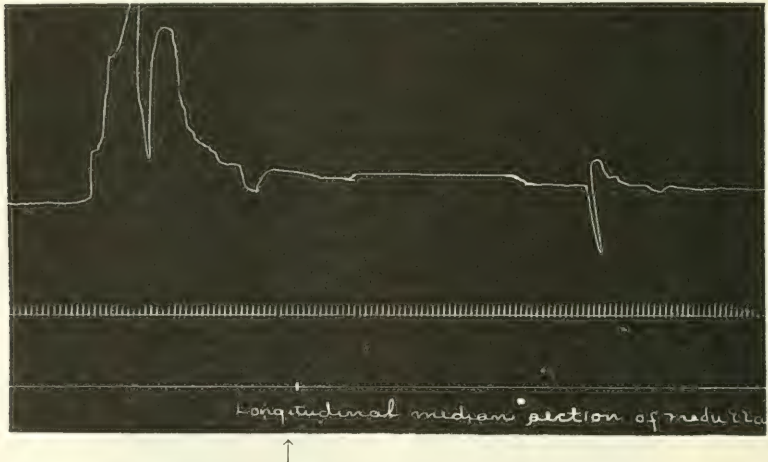


Fig. 3. Respiration after removal of the optic lobes, followed by longitudinal median section of medulla. (Continuation of fig. 2.)

It will be observed from the tracings that the form of the respiratory curves before and after decerebration was the same. The time intervals between respirations which, even under normal conditions were somewhat irregular, varying with the individual animal, were not appreciably altered by decerebration.

On the subsequent removal of the optic lobes, however, the character of the respiration underwent a marked change; the contractions of the respiratory muscles of the thoracic-abdominal cage, which in normal respiration were sudden, sharp and intense, became slow and shallow with greater intervals of time between each two successive respirations (fig. 3).

Following is a protocol of such an experiment.



*September 17, 1919*

10.00 a.m. Turtle etherized, cannula inserted in trachea and normal respiration recorded.

10.20 a.m. Decerebration; tracing of respiration.

10.36 a.m. Optic lobes removed; tracing of respiration.

11.00 a.m. Section through midline of medulla.

From a number of experiments performed in this manner it has become apparent that a median longitudinal section of the medulla, following removal of the optic lobes, results in an almost complete cessation of respiration. If, however, the optic lobes are intact, a

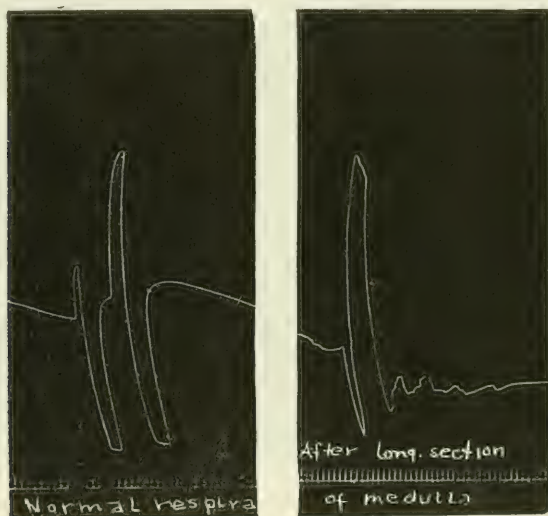


Fig. 4. Tracing 1, Normal respiration. Tracing 2, Respiration after longitudinal median section of medulla.

longitudinal median section has not such a severe effect. While the form of the respiratory curve may be somewhat altered, respiration proceeds quite adequately.

Several experiments were performed in which the first procedure was a longitudinal median section of the medulla oblongata, with subsequent removal of the optic lobes. Respiration continued until the optic lobes were separated from the brain stem, after which there were few or no respiratory movements (fig. 4).

Normal respiration is not altered by double vagotomy nearly as much as in the case of mammalia. Indeed, the conduct of the respira-

tion seems to indicate that in chelonians the greater part of the afferent impulses originate in the musculature concerned in the maintenance of respiration and are carried by the afferent spinal nerves to the medulla, rather than by the vagi. Moreover, vagotomy does not affect subsequent longitudinal median section of the medulla, as does ablation of the optic lobes (fig. 5).

H. Newell Martin (7) found in the frog, that "when the optic lobes are removed . . . such frogs breathe much less often than normal frogs or those with the corpora bigemina intact." From

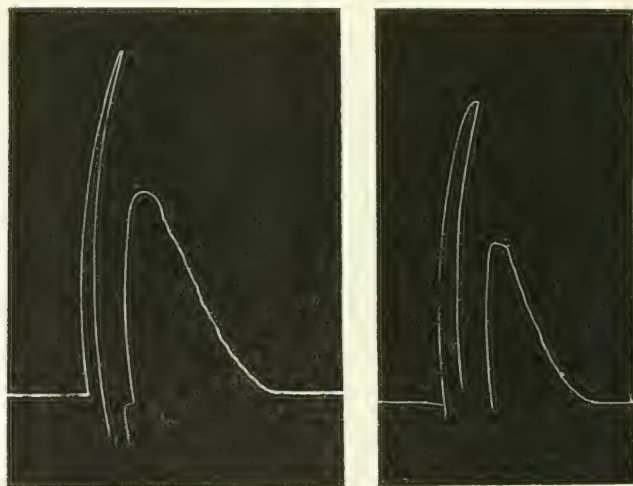


Fig. 5. Tracing 1, Normal respiration. Tracing 2, Respiration after double vagotomy.

the results of my experiments it appears to be true in the case of the turtle also; and as we ascend the phylogenetic scale, ablation of the optic lobes, or corpora quadrigemina in mammals, is attended by increasingly severe results (8).

In short, when we consider the evidence presented by the conduct of the respiration before and after the removal of the optic lobes, it seems to point to the existence in the optic lobes of nuclei auxiliary to the respiratory center in the medulla for the maintenance of normal respiration.

## CONCLUSIONS

1. Stimulation of the optic lobes or medulla produces contractions of the lungs in the turtle.

2. Since after vagotomy, stimulation of the optic lobes or the medulla elicits no contractions of the lung, the impulse to the lungs is carried over the vagus nerve.

3. Since a moderate stimulation of the optic lobes produces only contractions of the lungs and not inhibition of the heart, the nerve fibers in the vagus which pass to the lungs may have an origin anatomically different from those which pass to the heart.

4. Decerebration does not alter respiration in the turtle.

5. From the results obtained by stimulation and ablation of the optic lobes, it may be inferred that they contain nuclei auxiliary to the respiratory center in the medulla oblongata for the maintenance of normal respiration.

I desire to express my thanks to Prof. F. H. Pike of this department for his valuable suggestions and his kindness in criticising this work.

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## ON THE PROTECTIVE ACTION OF SOME ORGANIC SUBSTANCES ON CATALASE IN ACID MEDIUM

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The result described here is an objection to Burnett's report (1) which was published last summer. According to his report, the addition of a small amount of liver to muscle increases the catalytic activity of the mass, and blood also has an accelerating effect on the muscle catalase, nearly equal to that of liver. He explained this phenomenon as being due to an internal secretion of the liver, whereby the liver is supposed to send an activator to muscle and other tissues. My experiment has been performed for the purpose of studying *a*, whether his conclusion be true; *b*, how the so-called accelerating effect of the liver changes under various conditions of oxidation in the body and under various conditions of the liver. Satisfactorily as my results agreed with those of Burnett, yet I observed by further investigation that the apparent accelerating effect is chiefly due to the protective action of organic substances in muscle, not to a liver hormone, and what is more, the protective action is by no means specific.

### METHOD

As a test object the tissues of guinea pigs were used. The animals were killed and immediately perfused with an m/6 solution of sodium chloride from the aorta, until the fluid coming from the inferior cava was colorless. The leg muscles and other organs were then removed and the water remaining on them absorbed with blotting paper. The tissue and organs to be tested were ground up in a mortar. The pulpy mass thus obtained was weighed and extracted in an ice-chest for 12 hours with four times its volume of m/6 sodium chloride. The mixture was then centrifugalized and employed in volume quantities.

As a substrate 1 and 1.5 per cent solutions of hydrogen peroxide were used, prepared from commercial Sankyo's "Oxyful" with distilled



water, both in neutralized and unneutralized state. The oxygen was collected in a burette over water in the usual way, the amount given off in ten minutes being taken as the standard. The readings were all reduced to 0°C. and 760 mm. Hg. Three tests were made at a time, to avoid the influence of temperature, lighting, etc.

A. Experiment with 1.5 per cent acid hydrogen peroxide, the acidity of which amounts to  $n/200$ . In each test 50 cc. of solution were used.

1. The following table shows how the catalytic activity of the mass is increased by the combination of muscle and liver.

TABLE 1

| QUANTITY OF EXTRACT |             | OXYGEN LIBERATED |
|---------------------|-------------|------------------|
|                     | cc.         | cc.              |
| Muscle.....         | 5.0         | 1.9              |
| Liver.....          | 0.5         | 13.9             |
| Muscle.....         | 5.0 + liver | 153.1            |
|                     | 0.5         |                  |
| Muscle.....         | 5.0         | 1.9              |
| Liver.....          | 0.1         | 5.7              |
| Muscle.....         | 5.0 + liver | 58.9             |
|                     | 0.1         |                  |

2. Table 2 shows the comparison of catalytic activity, when muscle and other tissues were combined.

It may be suggested from the table that the combination of liver and muscle liberated most oxygen, and liver seems to have an accelerating action on the muscle catalase, as supposed by Burnett.

3. To test this supposition, the muscle extract heated for thirty minutes to 100°C. was used after filtration. This solution had no catalytic action on acid or neutral peroxide.

Table 3 demonstrates that the muscle extract, the existing catalase of which was entirely destroyed by heat, liberated, when small quantities of liver, spleen, adrenal gland, etc., were added, as much oxygen as the mixture of fresh muscle extract and other tissues. It is quite evident, therefore, that the liver does not accelerate the muscle catalase, but the accelerating agent is rather contained in muscle itself. As the table shows, the oxygen liberated is just proportional to the catalase content of the organs (cf. tables 2 and 3). These facts prove also that the muscle itself accelerates the catalase of the organs.

4. To test the specificity of the accelerating effect, a combination of liver and other organic substances was made.

TABLE 2

| QUANTITY OF EXTRACT      |                         | OXYGEN LIBERATED |
|--------------------------|-------------------------|------------------|
| cc.                      |                         | cc.              |
| Muscle.....              | 5.0                     | 1.9              |
| Liver.....               | 0.5                     | 13.5             |
| Muscle.....              | 5.0 + liver 0.5         | 153.1            |
| Muscle.....              | 5.0                     | 1.9              |
| Blood (20 per cent)..... | 0.1                     | 5.6              |
| Muscle.....              | 5.0 + blood 0.1         | 23.5             |
| Muscle.....              | 5.0                     | 1.9              |
| Adrenal gland.....       | 0.5                     | 8.0              |
| Muscle.....              | 5.0 + adrenal gland 0.5 | 53.5             |
| Muscle.....              | 5.0                     | 1.9              |
| Spleen.....              | 0.5                     | 0.0              |
| Muscle.....              | 5.0 + spleen 0.5        | 13.5             |
| Muscle.....              | 5.0                     | 1.9              |
| Kidney.....              | 0.5                     | 0.0              |
| Muscle.....              | 5.0 + kidney 0.5        | 11.2             |
| Muscle.....              | 5.0                     | 1.9              |
| Pancreas.....            | 0.5                     | 0.0              |
| Muscle.....              | 5.0 + pancreas 0.5      | 4.7              |
| Muscle.....              | 5.0                     | 1.9              |
| Testes.....              | 0.5                     | 0.0              |
| Muscle.....              | 5.0 + testes 0.5        | 3.8              |

TABLE 3

| QUANTITY OF EXTRACT | BOILED MUSCLE-EXTRACT | OXYGEN LIBERATED |
|---------------------|-----------------------|------------------|
| cc.                 | cc.                   | cc.              |
| Liver.....          | 0.5                   | 13.0             |
| Liver.....          | 0.5 + 10              | 113.1            |
| Adrenal gland.....  | 0.5                   | 7.6              |
| Adrenal gland.....  | 0.5 + 10              | 51.9             |
| Spleen.....         | 0.5                   | 0.0              |
| Spleen.....         | 0.5 + 10              | 5.9              |
| Pancreas.....       | 0.5                   | 0.0              |
| Pancreas.....       | 0.5 + 10              | 3.2              |
| Testes.....         | 0.5                   | 0.0              |
| Testes.....         | 0.5 + 10              | 2.7              |

*a.* Solution of egg white. Egg white was filtered through gauze and a 10 per cent solution made by adding an  $m/6$  solution of sodium chloride. It was then neutralized with  $n/5$  sulfuric acid. This solution had no catalytic action on acid or neutral hydrogen peroxide.

TABLE 4

| QUANTITY OF EXTRACT |       | SOLUTION OF EGG WHITE | OXYGEN LIBERATED |
|---------------------|-------|-----------------------|------------------|
|                     | cc.   | cc.                   | cc.              |
| Liver.....          | 0.5 + | 5.0                   | 125.4            |
| Adrenal gland.....  | 0.5 + | 5.0                   | 68.8             |
| Spleen.....         | 0.5 + | 5.0                   | 7.0              |

From table 4 it may be seen that the solution of egg white has also as much accelerating effect as the boiled muscle extract. This action is nearly proportional to the quantity of the solution of egg white.

TABLE 5

| QUANTITY OF LIVER-EXTRACT | SOLUTION OF EGG WHITE | OXYGEN LIBERATED |
|---------------------------|-----------------------|------------------|
| cc.                       | cc.                   | cc.              |
| 0.1 +                     | 10                    | 25.0             |
| 0.1 +                     | 20                    | 75.0             |
| 0.1 +                     | 30                    | 85.0             |

*b.* A 15 per cent solution of peptone was heated to 100°C. for thirty minutes and neutralized with  $n/5$  sulfuric acid. This solution had no catalytic action on acid and neutral peroxide.

TABLE 6

| QUANTITY OF EXTRACT |       | SOLUTION OF PEPTONE | OXYGEN LIBERATED |
|---------------------|-------|---------------------|------------------|
|                     | cc.   | cc.                 | cc.              |
| Liver.....          | 0.5 + | 5.0                 | More than 300.0  |
| Adrenal gland.....  | 0.5 + | 5.0                 | 238.7            |
| Kidney.....         | 0.5 + | 5.0                 | 52.1             |
| Liver.....          | 0.1 + | 5.0                 | 144.1            |
| Adrenal gland.....  | 0.1 + | 5.0                 | 44.6             |
| Kidney.....         | 0.1 + | 5.0                 | 3.1              |

It is surprising that the solution of peptone should have an accelerating effect far in excess of other substances!

*c.* Solutions of starch had no accelerating effect.

B. Experiment with 1 per cent neutral hydrogen peroxide. In each test 70 cc. of solution were used.

In the experiment with neutral hydrogen peroxide it was observed that the accelerating effect is much less manifested, and is sometimes even absent entirely.

TABLE 7

| QUANTITY OF EXTRACT                       | OXYGEN LIBERATED                  | INCREASE           |
|---|-----------------------------------|--------------------|
| cc.                                       | cc.                               | per cent           |
| Liver.....0.02                            | 22.5                              | ca. 60             |
| Muscle.....2.00                           | 10.3                              |                    |
| Liver.....0.02 + muscle 2.0               | 51.7                              |                    |
| Liver.....0.02                            | 29.1                              | ca. 70             |
| Muscle.....2.00                           | 13.6                              |                    |
| Liver.....0.02 + muscle 2.0               | 72.0                              |                    |
| Liver.....0.04                            | 89.2                              | ca. 20             |
| Muscle.....2.0                            | 14.3                              |                    |
| Liver.....0.04 + muscle 2.0               | 126.7                             |                    |
|   | OXYGEN LIBERATED<br>IN 20 MINUTES |                    |
| Liver.....0.02 (3 days after preparation) | 23.5                              | Over 100           |
| Blood.....0.02 (10 per cent fresh)        | 26.3                              |                    |
| Liver.....0.02 (10 + blood fresh) 0.02    | 125.8                             |                    |
| Liver.....0.05                            | 191.0                             | Slight retardation |
| Muscle.....0.00                           | 5.3                               |                    |
| Liver.....0.05 + muscle 1.0               | 181.0                             |                    |

These and many other similar experiments with a neutral hydrogen peroxide show the following facts:

1. A combination of any two tissues induces more or less of an increase of the catalytic activity of the mass. This effect is, however, generally much less manifest than when using acid hydrogen peroxide, and sometimes it is entirely absent.

2. The effect of boiled muscle or organ extract and peptone, etc., is similarly diminished when using neutral hydrogen peroxide.

3. The effect seems to disappear at a temperature lower than 14°C.

4. The effect seems to cease in case a relatively large amount of extract is used.



## DISCUSSION

The fact that the acceleration is especially manifested in acid medium, while it tends to disappear in a neutral medium, leads us to believe that some organic substance, such as protein, peptone or amino-acid, protects the catalase from destruction by the hydrogen ion in the acid hydrogen peroxide. Whether the mechanism is dependent on neutralization or protecting colloid, is not determined. Loevenhart (2) explained this phenomenon fifteen years ago as being due to a neutralization of acid by some substance contained in the tissue. Burnett contradicted this, however, and maintained that there was a more marked acceleration in neutral peroxide, taking the following example.

1. 0.5 gram of muscle + 0.02 gram of liver, acid  $H_2O_2$ , gave 170 cc. oxygen.

2. 0.5 gram of muscle + 0.02 gram of liver, neutral  $H_2O_2$ , gave 240 cc. oxygen.

This is, however, quite unreasonable. In test 1, with acid peroxide, 0.02 gram of liver and 0.5 gram of muscle give individually a very small amount of oxygen, so he may well say that the liberation of 170 cc. oxygen is marked acceleration. This is not the case with neutral peroxide. According to my experiment, 0.02 gram of liver may give more than 300 cc. oxygen in neutral peroxide, but in acid peroxide only 15 cc. It is therefore necessary to consider the amount of oxygen liberated by liver alone, in order to speak of "acceleration." All in all, there is no discussion about the fact that the apparent accelerating effect is no less than the manifestation of the activity of liver catalase protected by thermo-stable organic substance.

To ascertain the reason for the slight acceleration in neutral  $H_2O_2$  seems to be a rather difficult matter. Since the colloid substance is also increased in this case, the ideal of "protecting colloid" is naturally introduced thereby. On the other hand, the extract itself contains some hydrogen ions, which increase with the autolytic process. These H-ions also destroy the catalase, while the combination may protect it from injury. The addition of fresh blood to a relatively old extract increases therefore the activity most (cf. table 6).

## SUMMARY

1. The addition of a small amount of liver to muscle increases the catalytic activity of the mass markedly in acid hydrogen peroxide, while the effect diminishes or disappears in neutral hydrogen peroxide.

2. The accelerating effect is by no means specific. The combination of any two tissues induces more or less of an increase of catalytic activity. And what is more, the boiled muscle and organ extract, the solution of peptone and that of egg white, are as effective as the fresh extract.

3. The increase of catalytic activity is due to the protective action of some organic substance contained in muscle and organ, not to a liver hormone.

4. The mechanism of this protective action is not yet determined, but the idea of a neutralizing or protecting colloid is closely connected therewith.

The writer is indebted to Professor Doctor Mita for his kindly advice throughout the investigation.

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## GASTRIN STUDIES

### II.<sup>1</sup> FURTHER STUDIES ON THE DISTRIBUTION AND EXTRACTION OF GASTRIN BODIES

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In a previous article (1) the literature on the stimulating effects of organ extracts on gastric secretion was reviewed. It was shown that gastrin is uniformly distributed throughout the stomach; that it is found in much smaller concentrations in the duodenum, and that its presence in the esophagus could just be detected. Preparations of the pancreas, submaxillary gland, smooth muscle, striated muscle gave no gastric stimulation. The brain gave in two instances an increased flow of juice devoid of mucus and acid. The preparations were made by acid (0.4 per cent HCl) digestion of the tissues and the subsequent removal of the proteins by alcoholic precipitation. Popielski (2) contended that secretion in general is a function of the vascular and blood changes which can be brought about by tissue extracts in general. Collectively, he spoke of all of these secretory excitants extracted from tissues as *vasodilators*. Their solubility in alcohol constituted one of their properties. Therefore, to remove the vasodilating bodies our residues, after the removal of the proteins, were extracted three times with boiling absolute alcohol.

Rogers et al (3) digested tissues in alkaline physiological salt solution. The proteins were removed by heat coagulation in acid solution

<sup>1</sup> Article I—The distribution of gastrin in the body. This Journal, 1915, xxxvii, 481.

The term "gastrin bodies" as used by the authors includes the substance or substances found in tissue extracts which act as gastric secretagogues when injected intramuscularly.

and by salting out with ammonium sulphate. The residue then soluble in 95 per cent alcohol was used as the active preparation. Such preparations of thyroid, liver, parathyroids, spleen and pancreas showed marked activity. The activities of the thymus and pineal gland residues were much lower. Muscle preparations and Witte's peptone were inert. The authors suggested that the gastrin bodies are not confined to the mucosa of the gastro-intestinal tract, but are common to many extracts which influence favorably cellular nutrition and consequently the activity of the gastric epithelial cells, possibly through their nerve supply. They stated further that these stimulating residues acted through the peripheral nervous element upon some gastric secreting mechanism. The experimental work to substantiate this statement consists in the repetition of the experiments on doubly vagotomized animals without a change in results. This, of course, points to the mechanism being peripheral, but it does not necessarily mean that it is nervous.

In a more recent study the authors (4) have shown that the secretory response from thyroid residues can be much reduced by injecting adrenalin, atropin, nicotin and extracts of adrenal glands. Because atropin reduces the flow of juice from injection of thyroid residue and because of the established effect of thyroid extracts on metabolism these authors suggest that with the intermediation of the vagus or secretory nerve impulse the thyroid product increases the metabolism of the gastric epithelium. This view would then indicate that the vagus nerve is essentially trophic in its influence on the gastric cells. There is of course no experimental evidence presented by these observers that their extracts obtained from the thyroid really contained the stimulant to metabolism.

Since in our first studies we discarded the alcoholic extract in which Rogers and associates found their chief activity, it seemed advisable to repeat, with the modification suggested, our experiments on the tissues used in the previous report.

#### EXPERIMENTAL METHODS

Dogs with either gastric fistulae or Pawlow accessory stomachs were used for assaying the secretory strength of the preparations. In some of the animals either the splanchnic nerves or vagi had been sectioned. Our former experience had shown that the Pawlow stomach is a much less sensitive, but more reliable indicator of the gastrin content of a



tissue. It was hoped that by vagotomizing the animal with a gastric fistula an ideal test stomach could be obtained. This would give us secretion from the whole stomach and avoid the spontaneous secretion apt to be initiated through the vagus. The procedure resulted in a more refractory stomach which was by no means free from spontaneous secretion. Section of the splanchnics made the stomach much more responsive.

Acidities were determined by titration with dimethyl-amido-azobenzene (free acid) and phenolphthalein (total acid). These acidities were calculated to per cent of hydrochloric acid and all the figures given in the paper are thus reported. No attempt was made to measure the peptic activity. The degree of acidity and the quantity of juice are sufficient indicators of the state of the mechanism. Indeed, the concentration of pepsin always falls with an increase in flow of gastric juice although the total units of output are greater. So it alone does not furnish a convenient index of secretion.

One cubic centimeter of the extracts represented, as formerly, 4 to 5 grams of fresh tissues. They were prepared by the method already described (1). However the absolute alcohol extracts as well as the absolute alcohol insoluble residues were assayed as to activities after removing the alcohol and making up to volume in water.

*Alcoholic extracts.* As soon as the alcoholic extracts, which had been formerly discarded, were examined it was discovered that they were quite active. Indeed, it was further evident that the amount of activity was determined by the time and number of extractions.

This is shown in two protocols of experiments using extracts from stomach and duodenum.

*Dog I. Pawlow stomach, August 17, 1916. Injection of stomach extract*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS                      | PER CENT FREE HCl | PER CENT TOTAL HCl |
|-------|---|-------------------|--------------------|
| 9:25  | Dressed   |                   |                    |
| 10:25 | 0.6   | 0.00              | 0.02*              |
| 10:35 | Injected 1 cc. alcoholic extract of stomach intramuscularly |                   |                    |
| 11:35 | 2.1   | 0.07              | 0.11               |
| 12:35 | 0.2   |                   |                    |
| 12:35 | Injected 1 cc. residue after alcoholic extraction           |                   |                    |
| 1:35  | 3.5   | 0.16              | 0.21               |
| 2:35  | 0.4   |                   |                    |

\* The results of the titrations are calculated as per cent of hydrochloric acid.

*Dog IV. Gastric fistula, vagi crushed one year previously, August 23, 1916.*  
*Injection of duodenum extract*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS                           | PER CENT FREE HCl | PER CENT TOTAL HCl |
|-------|--|-------------------|--------------------|
| 9:00  | Dressed  | .                 |                    |
| 10:00 | 3.0  | 0.19              | 0.24               |
| 10:50 | Injected 1 cc. of alcoholic extract of duodenum                  |                   |                    |
| 11:50 | 15.8   | 0.35              | 0.40               |
| 12:50 | 0.8  | 0.31              | 0.38               |
| 12:50 | Injected 1 cc. of residue of duodenum after alcoholic extraction |                   |                    |
| 1:50  | 3.7  | 0.08              | 0.14               |
| 2:50  | 0.6  | 0.05              | 0.15               |

Each of these experiments was confirmed on two other animals, one having a very refractory Heidenhain stomach.

IS PEPTIC DIGESTION A FACTOR IN LIBERATING THE GASTRIN ACTIVITY?

Since appreciable peptic hydrolysis occurs in the preparation of the stomach extracts it was thought that possibly the extraction or liberation of the gastrin activity was facilitated in that case and that possibly peptic digestion of other tissues might yield more active preparations than simple extraction by 0.4 per cent HCl. Accordingly various tissues were finely divided, well mixed and separated into two portions; one to be extracted in the usual way with 0.4 per cent HCl and the other digested for five days at 35° to 40°C. in 0.4 per cent HCl with the addition of toluol and 5 grams of scale pepsin (1:3000 U. S. P.) per 250 to 350 grams fresh tissue. The other steps in the preparation were as previously described. Both were finally treated with alcohol in the usual way, but no extraction with absolute alcohol was carried out. The final solutions represented as before 4 to 5 grams fresh tissue per cubic centimeter.

As a control the amount of activity introduced by the pepsin had, of course, to be determined. Ten grams scale pepsin (1:3000 U. S. P. Armour) were dissolved in 0.4 per cent HCl, shaken with toluol, and digested at 35° to 40°C. for five days with frequent shaking. This was then heated to boiling, filtered and treated in the usual way. The final volumes were so concentrated that 1 cc. of the absolute alcohol soluble fraction was equivalent to 0.55 gram of pepsin and 1 cc. of absolute alcohol insoluble residue equal to 1 gram of the scale pepsin. A protocol attached shows that most of the activity goes into the absolute alcohol soluble fraction, but even this is not large.

*Dog I. Pawlow stomach, August 1, 1916*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS  | PER CENT FREE HCl | TOTAL HCl |
|-------|---|-------------------|-----------|
| 10:40 | Dressed   |                   |           |
| 11:40 | 1.0   | 0.00              | 0.03      |
| 11:55 | Injected absolute alcohol soluble fraction equivalent to 1 gram of scale pepsin (1 to 3000)   |                   |           |
| 12:55 | 2.4   | 0.14              | 0.17      |
| 1:55  | 0.5   |                   |           |
| 2:20  | Injected absolute alcohol insoluble fraction equivalent to 1 gram of scale pepsin (1 to 3000) |                   |           |
| 3:20  | 1.6   |                   | 0.03      |

A more refractory Heidenhain stomach gave just a trace of activity, while dog V (gastric fistula with vagi crushed one year previously) secreted from the entire stomach during the hour following the injection only 9 cc., free acidity 0.24; 0.32 total per cent HCl.

Reference to the long list of inactive preparations in table 4, in which the extracts injected corresponded to 0.01 gram, 0.07 gram and 0.1 gram of scale pepsin respectively, demonstrates clearly that any activity of the tissues extracted by the pepsin treatment is due to substances other than that introduced with the enzyme.

*Duodenum.* Reference to table 1 shows that when both absolute alcohol soluble and insoluble fractions are injected, the duodenal extract is quite as active as the stomach. The use of the scale pepsin does not appear to affect the activity of the preparation in any constant way; at any rate the comparative study suggests a loss or gain in activity, depending on which animal one considers. In five out of seven cases the stomach preparation was found more active than the duodenal extracts.

TABLE 1  
*Comparison of activity of gastric and duodenal mucosa*

| ANIMAL  | EXTRACT EQUIVALENT TO 4 TO 5 GRAMS<br>TISSUE | QUAN-<br>TITY* | ACIDITY                 |                          |
|---|--|----------------|-------------------------|--------------------------|
|   |  |                | Free<br>per cent<br>HCl | Total<br>per cent<br>HCl |
|   |  | cc.            |                         |                          |
| Dog I, Pawlow stomach   | Stomach                                      | 4.9            | 0.29                    | 0.34                     |
|   | Duodenum and HCl                             | 3.0            | 0.17                    | 0.25                     |
|   | Duodenum and pepsin + HCl                    | 2.5            | 0.25                    | 0.35                     |
| Dog II, Heidenhain<br>stomach                                 | Stomach                                      | 1.5            | 0.19                    | 0.26                     |
|   | Duodenum and HCl                             | 1.0            | 0.00                    | 0.05                     |
|   | Duodenum and pepsin + HCl                    | 1.4            | 0.09                    | 0.17                     |
| Dog III, Gastric fistula                                      | Stomach                                      | 7.5            | 0.33                    | 0.39                     |
|   | Duodenum and HCl                             | 7.3            | 0.19                    | 0.34                     |
|   | Duodenum and pepsin + HCl                    | 12.4           | 0.39                    | 0.46                     |
| Dog IV, Gastric fistula.<br>Vagi crushed 1 year<br>previously | Stomach                                      | 9.0            | 0.37                    | 0.41                     |
|   | Duodenum and HCl                             | 1.6            | 0.03                    | 0.08                     |
|   | Duodenum and pepsin + HCl                    | 6.5            | 0.22                    | 0.27                     |
| Dog V, Gastric fistula.<br>Vagi crushed 1 year<br>previously  | Stomach                                      | 15.0           | 0.46                    | 0.50                     |
|   | Duodenum and HCl                             | 10.5           | 0.41                    | 0.67                     |
|   | Duodenum and pepsin + HCl                    | 8.2            | 0.44                    | 0.50                     |
| Dog VI, Gastric fistula.<br>Vagi sectioned                    | Stomach                                      | 11.7           | 0.15                    | 0.43                     |
|   | Duodenum and HCl                             | 4.5            | 0.23                    | 0.42                     |
|   | Duodenum and pepsin + HCl                    | 15.5           | 0.14                    | 0.26                     |
| Dog VII, Gastric fistula.<br>Vagi sectioned                   | Stomach                                      | 5.2            | 0.30                    | 0.37                     |
|   | Duodenum and HCl                             | 6.2            | 0.26                    | 0.35                     |
|   | Duodenum and pepsin + HCl                    | 5.0            | 0.05                    | 0.13                     |

\*Quantity represents excess of secretion in experimental over control period.

*Liver.* The first extract of liver was prepared in August, 1916, and gave activity far above any crude preparations which we have studied. The pepsin digests appeared to be somewhat less potent than the simple acid extractions. These points are illustrated in the four protocols attached.



*Dog I. Pawlow stomach, September 19, 1916*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS   | PER CENT FREE HCl | PER CENT TOTAL HCl |
|-------|--|-------------------|--------------------|
| 7:55  | Dressed                                  |                   |                    |
| 8:55  | 0.5                                      |                   | 0.04               |
| 9:05  | Injected 1 cc. of liver extract (by HCl) |                   |                    |
| 10:05 | 11.0                                     | 0.43              | 0.047              |
| 11:05 | 6.0                                      | 0.47              | 0.51               |

*Dog I. Pawlow stomach, September 21, 1916*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS              | PER CENT FREE HCl | PER CENT TOTAL HCl |
|-------|---|-------------------|--------------------|
| 8:20  | Dressed   |                   |                    |
| 8:50  | 0.6   |                   | 0.09               |
| 8:50  | Injected 1 cc. of liver extract (by pepsin and HCl) |                   |                    |
| 9:50  | 6.5   | 0.39              | 0.44               |
| 10:50 | 1.0   | 0.34              | 0.40               |

*Dog VI. Vagotomized gastric fistula, September 19, 1916*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS   | PER CENT FREE HCl | PER CENT TOTAL HCl |
|-------|--|-------------------|--------------------|
| 8:25  | Dressed                                  |                   |                    |
| 8:55  | 4.5                                      | 0.33              | 0.47               |
| 9:10  | Injected 1 cc. of liver extract (by HCl) |                   |                    |
| 10:10 | 95.0                                     | 0.41              | 0.49               |
| 11:10 | 6.5                                      | 0.33              | 0.44               |

*Dog VI. Vagotomized gastric fistula, September 21, 1916*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS              | PER CENT FREE HCl | PER CENT TOTAL HCl |
|-------|---|-------------------|--------------------|
| 8:35  | Dressed   |                   |                    |
| 9:05  | 8.5   | 0.36              | 0.44               |
| 9:05  | Injected 1 cc. of liver extract (by pepsin and HCl) |                   |                    |
| 10:05 | 35.0  | 0.38              | 0.46               |
| 11:05 | 4.0   | 0.29              | 0.41               |

These results were confirmed on five other animals in a series of twenty-three experiments. Their uniformity was quite striking. An attempt was made to confirm these results on liver extracts made on October 26 and November 11 of the same year. The first of these stood for two days in the ice-box and the second one was made immediately

upon receipt of fresh liver. On the two preparations of October 26, (one HCl extract, the other HCl and pepsin extract) four experiments were conducted. Reviewing the behavior of these animals we regarded one experiment as positive, two as showing a trace of activity and the fourth negative. The positive experiment is cited to show the low grade of activity as compared with experiment on same animal on September 19.

*Dog I. Pawlow stomach, November 9, 1916*

| TIME               | QUANTITY OF JUICE IN CUBIC CENTIMETERS   | PER CENT FREE HCl | PER CENT TOTAL HCl |
|--------------------|--|-------------------|--------------------|
| Secretion 1 hr...  | 1.5                                      |                   | 0.08               |
|                    | Injected 1 cc. of liver extract (by HCl) |                   |                    |
| Secretion 1st hr.. | 2.9                                      | 0.09              | 0.16               |
| Secretion 2nd hr.  | 1.0                                      | 0.14              | 0.21               |

On the preparations of November 11, five experiments were run using three Pawlow stomachs, two of these being quite sensitive. Dog IX had both splanchnic nerves cut as they emerge from the thorax beneath the pillars of the diaphragm. The two more sensitive animals responded, but the other one (dog VIII) gave negative results. The activity was somewhat greater than in the extracts of October 26, but was in no way comparable to results on the August preparations.

TABLE 2  
*Activity of pancreas preparations*

| ANIMAL | TYPE OF STOMACH                                      | DATE AND METHOD OF EXTRACTION |            |            |            |             |            |
|--------|--|-------------------------------|------------|------------|------------|-------------|------------|
|        |  | August                        |            | October 26 |            | November 26 |            |
|        |  | HCl                           | HCl pepsin | HCl        | HCl pepsin | HCl         | HCl pepsin |
| I      | Pawlow.....  | —                             | +          | —          | +          | —           | —          |
| II     | Heidenhain.....                                      | —                             | +          | —          | —          |             |            |
| III    | Gastric fistula.....                                 | —                             | +          | —          | —          |             |            |
| IV     | Gastric fistula, vagi crushed 1 year previously..... | —                             | +          | —          | —          |             |            |
| V      | Gastric fistula, vagi crushed 1 year previously..... | ?                             | +          | —          | ?          |             |            |
| VI     | Gastric fistula.....                                 | —                             | +          |            |            |             |            |
| VII    | Gastric fistula, vagotomized.....                    | —                             |            |            |            |             |            |
| VIII   | Pawlow.....  |                               |            | —          | —          |             | —          |
| IX     | Pawlow, splanchnics cut.....                         |                               |            | —          | ?          |             | +          |

*Pancreas.* Reference to table 2 shows that the August preparation with pepsin extraction was uniformly active on all animals. In only one case (dog V) was there any trace of activity in the HCl extract. The other preparations of October 26 and November 16 gave two positive and two questionable reactions out of seventeen experiments.

An idea of the activity of extracts may be secured from the attached protocols.

*August preparations*

*Dog I. Pawlow stomach, September 24, 1916*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS                 | PER CENT FREE HCl | PER CENT TOTAL HCl |
|-------|--|-------------------|--------------------|
| 9:50  | Dressed  |                   |                    |
| 10:50 | 1.6  | 0.00              | 0.04               |
| 11:10 | Injected 1 cc. of pancreas extract (by pepsin and HCl) |                   |                    |
| 12:10 | 5.7  | 0.33              | 0.37               |
| 1:10  | 0.5  | 0.46              | 0.55               |

*Dog IV. Gastric fistula, vagi crushed one year previously,  
September 24, 1916*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS                 | PER CENT FREE HCl | PER CENT TOTAL HCl |
|-------|--|-------------------|--------------------|
| 9:50  | Dressed  |                   |                    |
| 10:50 | 0.2  |                   | 0.06               |
| 11:10 | Injected 1 cc. of pancreas extract (by pepsin and HCl) |                   |                    |
| 12:10 | 34.6   | 0.43              | 0.47               |
| 1:10  | 4.6  | 0.40              | 0.42               |

With these may be compared the positive experiment on dog I with the preparation of October 26.

*Dog I. Pawlow stomach, November 8, 1916*

| TIME  | QUANTITY OF JUICE IN CUBIC CENTIMETERS              | PER CENT FREE HCl | PER CENT TOTAL HCl |
|-------|---|-------------------|--------------------|
| 9:30  | Dressed   |                   |                    |
| 10:30 | 1.6   |                   | 0.09               |
| 10:30 | Injected 1 cc. pancreas extract (by pepsin and HCl) |                   |                    |
| 11:30 | 2.5   | 0.09              | 0.12               |

The other positive experiment was on the preparation of November 16, the animal used being an extremely sensitive Pawlow stomach, the splanchnic nerves of which had been sectioned.

*Thyroid.* Consideration of table 3 reveals the fact that thyroid extracts give a rather definite activity by our method. These preparations were made in October under the conditions which had resulted in negative extracts of liver and pancreas. It is further evident that the pepsin does not add to or detract from their activity since in three animals (I, III, VI) the HCl extract was more potent; in two (IV, V) the pepsin and HCl was the more active; in another (VIII) there was no difference; and lastly, dog VII gave results diametrically opposed at various times.

The variability in results is due to the state of the stomach at the time of experiment. As every one knows, this is such a variable factor that the assay of activity frequently requires many repetitions.

TABLE 3  
*Activity of thyroid extracts*

| ANIMAL   | PREPARATION    | INCREASE<br>IN QUAN-<br>TITY<br>JUICE* | PER CENT ACID IN<br>HOUR FOLLOWING<br>INJECTION |       |
|--|----------------|--|---|-------|
|  |                |  | Free  | Total |
|  |                | cc.                                    |   |       |
| Dog I, Pawlow stomach.....                     | HCl            | 3.5                                    | 0.18  | 0.26  |
|  | HCl and pepsin | - 0.4                                  | 0.13  | 0.23  |
| Dog III, Gastric fistula.....                  | HCl            | 17.5                                   | 0.24  | 0.29  |
|  | HCl and pepsin | 1.6                                    | 0.02  | 0.15  |
| Dog IV, Gastric fistula, vagi<br>crushed.....  | HCl            | 6.5                                    | 0.29  | 0.36  |
|  | HCl and pepsin | 20.0                                   | 0.36  | 0.42  |
| Dog V, Gastric fistula, vagi<br>crushed.....   | HCl            | 14.0                                   | 0.38  | 0.42  |
|  | HCl            | 19.0                                   | 0.49  | 0.52  |
|  | HCl and pepsin | 26.4                                   | 0.45  | 0.50  |
| Dog VI, Gastric fistula, vagoto-<br>mized..... | HCl            | 7.6                                    | 0.10  | 0.12  |
|  | HCl and pepsin | - 0.4                                  | 0.18  | 0.27  |
| Dog VII, Vagotomized, gastric<br>fistula.....  | HCl            | 3.7                                    | 0.16  | 0.24  |
|  | HCl and pepsin | - 0.5                                  | 0.11  | 0.22  |
|  | HCl and pepsin | 7.9                                    | 0.40  | 0.46  |
| Dog VIII, Pawlow.....                          | HCl            | 3.6                                    | 0.27  | 0.34  |
|  | HCl and pepsin | 3.6                                    | 0.20  | 0.36  |

\* This quantity was determined by subtracting the quantity secreted in 1 hour control period from the first hour experimental period. The (-) sign indicates control output was larger in control than experimental period.



*Negative extracts.* Table 4 contains a condensed summary of experiments on brain, muscle, spleen, thymus, peptone mixture and gastric juice. The table and the individual protocols show that these preparations do not influence the secretory activity of the stomach.

TABLE 4  
*Negative extracts and preparations*

| TISSUE   | NUMBER<br>OF EX-<br>PERI-<br>MENTS | NUMBER<br>OF ANI-<br>MALS | NEGATIVE | QUESTION-<br>ABLE | POSITIVE |
|--|------------------------------------|---------------------------|----------|-------------------|----------|
| Brain.....   | 13                                 | 8                         | 10       | 3                 | 0        |
| Muscle.....  | 15                                 | 8                         | 15       | 0                 | 0        |
| Spleen.....  | 10                                 | 6                         | 7        | 2                 | 1        |
| Thymus.....  | 12                                 | 6                         | 12       | 0                 | 0        |
| Gastric juice.....   | 19                                 | 8                         | 19       | 0                 | 0        |
| Fibrin pepsin HCl digest .....   | 4                                  | 4                         | 4        | 0                 | 0        |
| Filtrate from the lead acetate precip-<br>itate of fibrin pepsin HCl digest... | 4                                  | 4                         | 4        | 0                 | 0        |

*Gastric juice.* The gastric juice was collected under varying conditions of stimulation. Thus samples were obtained from Pawlow stomachs under food and gastrin stimulation, from gastric fistulae under spontaneous secretion and from human stomach (Mr. V. reported by Carlson (5) ) following the chewing of food. The juice (1 cc.) was injected without concentration in some cases (3 experiments); in the other cases, the amount injected represented from 4.7 to 11.5 cc. original gastric juice. Animals with gastric fistulae and Pawlow stomachs were used to assay the preparations. In no case was there a positive response.

*Fibrin peptone proteose mixture.* Popielski (2) has called attention to Witte's peptone as a source from which his vasodilators could be prepared. It seemed important to investigate this as well as the tissues previously discussed. Instead of starting with a commercial preparation whose history was unknown, a fibrin digest was selected. In this way we could be certain that any activity developing must be due to some of the hydrolytic products and not to substances extraneously introduced. The protocol of this preparation follows.

Beef fibrin was washed free from blood in flowing water, pressed in cloth and the net weight (175 grams) was determined. This was next suspended in 7000 cc. of 0.2 per cent sodium hydroxide for six days, strained and filtered. To the opalescent filtrate was added one volume water and sufficient of a 0.5 per cent acetic acid solution to cause a good flaking out of the fibrin. This latter was allowed to settle, washed

by decantation with distilled water four times, using 8 liters for each washing. Finally, it was filtered by suction and moist weight (206 grams) determined. This constituted the purified fibrin, the substrate from which the peptone was made.

Of this substrate, 103 grams moist weight, (15 grams dry weight) was mixed with 300 cc. of 0.4 per cent HCl and 25 cc. of a 0.1 per cent solution U. S. P. pepsin (Armour's) preserved with toluol and incubated at 35° to 40°C. After three days, 5 cc. more of a 1.66 per cent solution of same pepsin was added; the mixture was incubated another 24 hours, filtered, and the filtrate concentrated in vacuo to 50 cc. The filtrate was then precipitated with 300 cc. of redistilled (95 per cent) alcohol, the precipitate removed and the filtrate again concentrated in vacuo to dryness. Alcohol was removed, the residue dissolved in water and diluted to 25 cc. One cubic centimeter of this solution represented about 4 grams by weight of the fresh material and approximately 0.004 gram of scale pepsin. This constituted the test solution of proteose-peptone which was injected into the animals.

Reference to table 4 shows that the experiments all gave negative results. This solution was further precipitated by basic lead acetate and after the removal of the lead from the filtrate tested against the same animals. This procedure was followed because in the course of studies on purification of gastrin from stomach mucosa the active principle had been found in the filtrate from the basic lead acetate precipitate. These experiments also showed no activity.

We may conclude that an acid pepsin digest of purified fibrin gives no products which are capable of causing gastric secretion when injected intramuscularly.

#### DISCUSSION

*Solubility of gastrin bodies in alcohol.* Our experiments demonstrate that the gastrin preparations exhibit rather definite evidence of vasodilatation as shown by the reddening of the nose and buccal mucosa of the experimental animals. We have not taken records of the blood pressure on intravenous injection. However, we published a tracing (1) showing that the blood pressure was not appreciably reduced when the injection was made into the muscles of the animal and we called attention to the fact that the maximum period of secretion came after the evidences of vasodilatation had subsided. In the data just presented it is clear that absolute alcohol cannot be relied upon to separate

the two physiological activities, i.e., the vasodilatation observed after intravenous injection and the secretagogue action resulting from intramuscular injection or, in other words, the vasodilators of Popielski from the gastrin bodies, if they really be different substances.

Formerly we concluded that the duodenal preparations presented a secretory activity definitely less than similar preparations from the stomach. When however we combined the alcohol soluble fraction, previously discarded as containing vasodilators, the activity of the two tissues was the same. Maydell (6) states that if secretin is treated with alcohol and ether it is possible to separate the two component parts of gastric secretin, one of which evokes secretion and the other dilates vessels. Up to date we have been unable to secure his original report and for this reason cannot discuss the results further than to quote the words of the abstractor.

Tomaszewski (7) extracted the residue of his preparation (stomach mucosa extract dried at 90°) with absolute alcohol. Practically all of the active substance was removed from the insoluble residue by this treatment, but only about one-seventh of the original activity could be found in the filtrate. A precipitation of proteins by six volumes absolute alcohol on the contrary appeared to increase the activity in the filtrate. This he attributed to a physical change brought about in the mixture, perhaps freeing the body in question more definitely from the proteoseptone molecules. Again, he precipitated the acid preparation with six volumes of alcohol, the residue was discarded and then a second precipitation was made upon the filtrate after reconcentrating. After three such treatments the activity in the last filtrate was almost completely lost. This experiment only means that the major portion of the active substance must have been carried over in the precipitates and thus disappeared. We conclude that these experiments furnish satisfactory confirmation that gastrin bodies are difficultly soluble in absolute alcohol.

*Relation of gastrin bodies to the vasodilators of Popielski.* It is our belief that it cannot be determined at present whether the power of stimulating the gastric mucosa and of causing vasodilatation are two properties of the same substance, or whether they are to be attributed to bodies of different chemical structure. However, Tomaszewski's (7) experiments are of interest at this point. He confirms our former statement that intravenous injections of known active extracts gave little or no secretory response, but a maximum of toxic manifestations. Such a secretion as results he believes is purely a mechanical squeezing out of



residual juice that may have been present. Thus, in addition to his extracts he found that 20 cc. of 5 per cent Witte's peptone solution also gave a small secretion when introduced intravenously, but no secretion at all when injected subcutaneously. Therefore he considers that there are two separate mechanisms involved. The one is elicited by intravenous injections and is perhaps due to mechanical factors, blood alterations and vascular changes. This is the secretion due to vasodilatation and the secretion which he claims will be given by any proteose-peptone solution as, for example, Witte's peptone. The other is elicited on the subcutaneous injection of a body that is a real secretory excitant. Tomaszewski also repeated the experiments of Edkins on cats, but he was able to get only an insignificant increase in acidity of the sodium chloride solution introduced into the stomach. This result he believes is to be explained as a reaction of the first type and that therefore Edkins has in no sense demonstrated the existence of a gastric hormone.

*Effect of pepsin on the extraction.* It is possible that the pepsin and HCl digestion occurring in the process of preparing the stomach extracts may be a factor in the production of the active substance, either liberating it through the more thorough breakdown of the tissues or through the actual hydrolysis of a particular complex. In other words, the cleavage of the molecule by pepsin may be significantly different from the simple HCl digestion, and to this difference the activity of the preparations may be attributed. However, our experience shows that pepsin does not influence the extraction in any constant fashion. Thus it appears to increase the activity in the cases of duodenum and pancreas, to lessen it in the liver preparations and not to influence it in the case of the thyroid. However, Tomaszewski has published one experiment showing that incubation of an active extract with gastric juice for two days reduces its activity to one-third the value of the original. He concludes that pepsin may destroy this class of bodies, but trypsin does not.

*Distribution of gastrin activity.* Our results indicate that the stomach, duodenum and thyroid have the same concentration of active substance per gram of fresh tissue.

The liver and pancreas showed activity in one preparation far above any other tissue extracts, but the activity was practically absent in other samples prepared some months later. We are unable to explain this result at present. However, we will have occasion to show later that there are at least two classes of substances which produce secretory



activity. These we know behave differently in their precipitations. Gastrin is extremely stable in acid media, but the stability of the other class of substances has not been investigated. It is possible, therefore, that in the case of the pancreas and liver the activity may be due to this second class of substances. The readiness with which these two tissues autolyze and the ease with which they may be converted into excellent bacterial media may explain the formation of this second group of active substances. Finally, the liver is an extremely mobile metabolic organ and variations in the nutritional state of the animals might explain the discrepancy. Whatever may be the explanation, our method did not give uniformly active preparations from these tissues.

Spleen, thymus, brain, muscle, gastric juice and fibrin peptone-proteose digest were uniformly negative. These results differ from Rogers and associates who found the spleen, pancreas and liver active, with thymus showing a smaller concentration of the active substances. It must be recalled that these investigators used an alkaline physiological salt solution for their extraction while we were using 0.4 per cent HCl. This of course does not mean that bodies possessing secretory activity may not occur in these tissues. It simply indicates that our method which has uniformly given active preparations from stomach and duodenum gives such an active preparation from the thyroid, but not a uniformly active one from liver, pancreas, spleen, thymus, brain, striated muscle, gastric juice and fibrin digest.

It is an interesting question whether the active substance is preformed in the tissues and merely liberated by the acid digestion or whether it is a product resulting from the acid hydrolysis of protein. We have some evidence suggesting the latter to be true. Some of our earliest observations not reported up to present, are to the effect that water extracts of well dried gastric mucous membrane freed from fats are not active as secretagogues. The fact that gastrin is present uniformly in some tissue digests and not in others does not rule out the possibility of its being a building stone in certain proteins. If it be an extractive then its physiological possibilities assume greater interest. The absence of this substance or these substances from the gastric juice should be emphasized. We will have occasion to return to this point in a later communication.

In view of our other experiments attempting to purify the stomach preparation, it seemed inadvisable to pursue further the question of distribution of gastrin bodies until we knew more of their chemical behavior and nature.

## SUMMARY

1. Gastrin bodies are soluble in absolute alcohol. Therefore the method of absolute alcohol extraction cannot be used in separating the vasodilators of Popielski from them.

2. When an extract corresponding to 1 gram of Armour's scale pepsin (1/3000) is injected, a slight secretory activity results. Secretion does not occur after an injection of 0.10 gram pepsin, the quantity used in the peptic digestion of the various tissues.

3. The stomach and duodenum contain approximately the same concentration of gastrin bodies.

4. A liver preparation made at one time gave an activity far in excess of the extracts from any other tissues. Similar extracts, prepared later, were practically inactive.

5. The pancreas extracted with pepsin and hydrochloric acid (on one occasion) gave more activity than the stomach and duodenum, but less than the liver. The hydrochloric acid extract at the same time was inactive. Similar preparations on another occasion (two months later) were inactive. The reason for this difference has not been determined.

6. The thyroid preparations presented about the same order of activity as the stomach and duodenum.

7. The spleen, thymus, brain, muscle, gastric juice and fibrin peptone-proteose preparations were uniformly inactive.

8. Pepsin and hydrochloric acid is not a better medium for extraction than hydrochloric acid alone.

9. The existence of two classes of bodies causing gastric secretion is suggested. Whether these bodies are extractives from special tissues or hydrolytic cleavage products has not been determined. The investigation in the distribution of gastrin bodies has been temporarily abandoned for the more promising studies into the chemical nature of the product derived from the gastric mucosa.

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## PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE

### I. ON THE LUCIFÉRINE AND LUCIFÉrase OF CYPRIDINA HILGENDORFII<sup>1</sup>

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#### INTRODUCTORY

Raphael Dubois (2) found in 1885 that two substances are concerned in light production of the West Indian cucullo, *Pyrophorus noctilucus* and also of a luminous mollusc, *Pholas dactylus*. He called one of these light-producing substances "luciférine," which is not destroyed by boiling, and the other "luciférase," which is destroyed by boiling and was assumed to be an oxidizing enzyme.

E. Newton Harvey who investigated the mechanism of light production in luminous bacteria, fire-flies and others, "believed Dubois' interpretation" of these experiments to be correct. But after he studied the light production of a Japanese ostracod crustacean, *Cypridina hilgendorffii*, he was led "to wholly different conclusions regarding the existence of luciférine and luciférase" (6, p. 322). He has proposed new views concerning the light-producing substances and has adopted new words, "photophelein" and "photogenin" for Dubois' luciférine and luciférase.

The writer has attempted to test Harvey's conclusions with *Cypridina hilgendorffii* and has found many experimental facts essentially contradictory to those of Harvey and rather in accordance with those of Dubois.

#### MATERIAL AND COLLECTION

The material used by the writer for all of the following experiments was the same species of ostracod crustacean, *Cypridina hilgendorffii*<sup>2</sup>

<sup>1</sup> A preliminary report of this work was published in the Japanese Journal of Zoölogy, 1918, xxx, 409, 445.

<sup>2</sup> The writer's thanks are due to Dr. Naohide Yatsu for identification of the animal.



used by Harvey, which is abundant in Tsuyazaki gulf, Japan. It is caught the year round but is most abundant during April and November.

The animal in general is strongly negatively heliotropic, so that it is best collected at night by means of a porcelain jar about 30 cm. high and 20 cm. in diameter. The head of a shark (on which the animal will feed) is placed in the jar, which is covered by a piece of cloth with a small hole in the center. Ten or more of such jars may be used in series connected with a long rope. These jars are submerged to the bottom of the sea about 15 feet deep. The animals go into the jars being attracted by the smell of the fish heads, and stay there feeding. This is a very striking case of chemotropism. In this way large quantities of the animals are readily collected.

Harvey states that the animal is not readily caught on moonlight nights on account of its negative heliotropism. If, however, the method just mentioned above is adopted, no difficulty is encountered even on moonlight nights. The positive chemotropism of the animal is much stronger than its negative heliotropism. Harvey also states that "another non-luminous species (*Cypridina x*) is often obtained from the fish heads together with *C. hilgendorffii*. It is positively heliotropic to lamp-light" (6, p. 319). "Non-luminous *Cypridina x*" is, however, not caught at Tsuyazaki gulf, so far as the writer's experience goes; but some individuals of *Cypridina hilgendorffii*, and sometimes many of them, are found to be positively heliotropic to strong daylight.

#### THE MAXILLARY GLAND AND LUMINOUS SECRETION OF CYPRIDINA HILGENDORFFII

A full account of the maxillary gland and its luminous secretion will be found in the papers of Harvey (6) and Yatsu (8). It is, however, not out of place to call special attention to the fact that Müller, the discoverer of this species, first pointed out the presence of "two groups of gland cells of different nature" with different secretion products. "Furthermore, he advanced the view that light is produced by the interaction of these two substances" (8, p. 438. Watanabe also states that the maxillary gland secretes a colorless transparent fluid and a yellow homogeneous substance (7, p. 87). Yatsu has especially emphasized these points in his paper based on his histological studies (8), (though Harvey has entirely overlooked them); particularly "the presence of two kinds of gland cells and the absence of a reservoir for the secretion granules common to all the gland cells" (8, p. 438).

Special attention is called to the fact, which has been made clear by the statements referred to above, that the animal produces no light in the maxillary gland cells but the light appears outside of the body of the animal when the light-producing substances secreted by the cells meet in the sea water. In other words, *Cypridina hilgendorffii* has in no sense any luminous organ or organs as have many other animals, fireflies, for example. In the writer's opinion, therefore, Harvey was entirely misled on this point when he uses such phrases as "the luminous parts" or "non-luminous parts of *Cypridina hilgendorffii*," "the luminous organs of *Cypridina*," "the luminous gland" or "luminous gland cells" and so forth all the way through in his paper (6). This point should be remembered when the distribution of the light-producing substances is discussed later on.

#### THE PRESERVATION OF THE MATERIAL

For various reasons, living Cypridinas are not good material to use for experimental work. The writer, therefore, always used dried animals, except for some special purposes.

Harvey states that he dried Cypridinas over  $\text{CaCl}_2$  (6, p. 321). But this is an extremely slow process and the light-producing substances are not often in a satisfactory condition, most probably being injured by moisture in the course of the drying time. The writer adopted, therefore, the following rapid method, which proved satisfactory, as table 2 will show. He made a wire ring to which white cloth was fastened. The animals taken out of the sea water were placed on the cloth. The water on the animals was then removed by absorbing it in blotting-paper as completely as possible. After this they were spread on sheets of dry blotting-paper and exposed to direct sunlight. They were stirred up from time to time in order to dry them evenly. At 30 to 35°C., they could be thoroughly dried in a few hours. If the animals thus dried are placed in a desiccator with  $\text{CaCl}_2$  they may be kept over eight months without impairing their power to produce light when again moistened.

Harvey states that removal of the animals "from sea water also inhibits the ejection of the secretion" (6, p. 320). But this statement is not quite correct. In the course of drying, the animals may live for thirty minutes or longer even in direct sunlight (the time depending upon the temperature) and continue to eject the secretion which is readily observed by the naked eye, as judged by the color. The light-producing substances, therefore, may be lacking in some animals, so

that they may not be of much use when they are finally dried. The quicker the drying, therefore, the better the method, beyond doubt.

The animals dried with the method just mentioned may also be preserved as a whole in pure ether. Or they may be crushed and sifted with a proper sized sieve in order to separate bodies and shells; and then the bodies, either as they are, or after extracting the fatty substances by several changes of ether during the course of a few days, may be preserved in pure ether as experimental material for as long as eight months or more. Of alcohol, ether and chloroform as preservatives, ether is the best owing to its low specific gravity. The material readily sinks into it. This is not the case with chloroform. Moreover, ether is most convenient to work with because of its quick evaporation when the material is taken out of it.

#### THE SEPARATION OF THE MAXILLARY GLAND

On account of its minute size it is next to impossible, if not absolutely impossible, to dissect out the maxillary gland by itself. At any rate, the writer confesses that he has not succeeded in this attempt. Nevertheless, he believes that the following method is an improvement upon that of Harvey (6, p. 325):

Under a lens the anterior part of dried crushed Cypridina where the maxillary gland is located, is carefully dissected out with one pointed dissecting knife in each hand. But it is hardly necessary to mention that other substances besides the light-producing substances are also cut out. The dissected gland cells examined under a low power of the microscope are very dark, though they are yellow while living. The dissected anterior parts, however, cannot be kept very long in contact with air, though they may be preserved a few days in a vacuum desiccator without impairing the power of light production. The light-producing substances are undoubtedly destroyed by moisture or oxygen or by both.

#### THE EXISTENCE OF LUCIFÉRINE AND LUCIFÉRASE

As already stated, the existence of two different substances necessary for the production of light by Cypridina was pointed out by Müller, Watanabe and Yatsu on the basis of morphology. Harvey also demonstrated this fact experimentally in Cypridina, as Dubois did in a beetle and a mollusc. Unfortunately, however, the latter

two investigators differ completely in their interpretation, although the results seem essentially similar in nature. At any rate, the fact that the existence of two different substances is concerned in the light production of Cypridina, is readily demonstrated in the following way.

About twenty of the dried individuals are placed in 50 cc. of distilled water. Immediately after this treatment, a brilliant light ensues. After a few hours' standing, the mixture is filtered through heavy filter-paper and the filtrate, which gives no light by itself any longer,<sup>3</sup> is kept for testing. For convenience's sake, this water extract may be called "A" solution. On the other hand, another set of twenty individuals are put into 50 cc. of boiling distilled water.<sup>4</sup> No light results in this case. After a few minutes of boiling, the cooled mixture is filtered and the filtrate (which produces no light by itself) is kept for testing. This may be called "B" solution. If now a small amount of "A" solution is added to a large amount of "B" solution, a brilliant light results at the moment and place of the contact of the two solutions; and the light spreads all over. Its brilliancy does not, however, last very long, but it remains dim for many hours to trained eyes in a dark room.

This phenomenon may be explained on the hypothesis that two light-producing substances are secreted respectively by two different kinds of cells in the maxillary gland. At any rate, that the presence of two different substances is concerned in the production of light by the animal is obvious. That is to say, one of these two substances is destroyed by boiling while the other is not. This thermolabile substance which may be temporarily called X substance, therefore, is left in the "A" solution and the thermostable one, which may be called Y substance, is in the "B" solution intact. The mechanism is this: When the mixture of the material and water is allowed to stand, all the Y substance is used up and the X substance is left in the mixture. On the other hand, when the mixture is boiled the X substance is destroyed while the Y substance is left intact, because the former is destroyed by heat before it can use the latter for light production. On this view, the Y substance is "the source of the light" and the X

<sup>3</sup> Light may be observed by trained eyes even after ten hours, though faint. Harvey might have mistaken it as light produced when this solution was mixed with many chemical substances.

<sup>4</sup> The beaker for boiling water should be tall and the flame has to be lowered after the water has reached the boiling point. No material must stick on the wall of the beaker when the material is put in.



substance is something which causes the former to emit light, as already stated. This conclusion is opposed to that of Harvey (6, p. 323) and is similar theoretically to that of Dubois.

According to Harvey, the substance destroyed by boiling "possesses certain properties . . . characteristic of enzymes" (6, p. 323). But it can not be regarded as an enzyme because it is "slowly used up" in the reaction (6, p. 324). He, therefore, invented a new word, "photogenin," to indicate the "light producer" for Dubois' luciférase. And this, claimed Harvey, is "the source of the light" (6, p. 323). In the writer's opinion, however, it is hasty to conclude that the substance is "used up" when it slowly disappears in the reaction, because it may not be "used up" in the reaction, but its nature may be changed due to the instability of the enzyme itself. It is a well-known fact that a solution of any enzyme loses its activity if kept. "This is, in great part, due to the complex colloidal state of these substances" (1, p. 313). On this view, then, the disappearance of the thermolabile substance furnishes strong evidence of an enzyme, although Harvey declares to the contrary. The writer, therefore, could find no objection in using Dubois' luciférase to indicate the nature of the action of the thermolabile or X substance found in the gland cells of Cypridina. For this reason he will hereafter use the word "luciférase" to indicate the X substance.

As to the Y substance which is not destroyed by boiling, Harvey names it "photophelein" for Dubois' luciférine, that is the "light assistor" (6, p. 324). This "is something which causes the luciférase to emit light" (6, p. 323). As already pointed out, however, the writer regards this substance as the source of the light, contrary to Harvey's interpretation. The writer therefore defends Dubois' luciférine, as he thinks it adequate to indicate the light-producing nature of the thermostabile substance.

As far as the interpretation of the facts and the phraseology of the light-producing substances are concerned, Dubois and the writer are quite in agreement with each other. According to the former, however, the luciférase of *Pholas* is an oxidizing enzyme while the luciférine is capable of oxidation, with light production, by means of the luciférase. Since the writer has recently found that the phenomenon of light production of dried crushed Cypridinas is by no means an oxidation,<sup>5</sup> he can neither hold the luciférase of Cypridina as an oxidizing enzyme nor the luciférine as a substance capable of giving

<sup>5</sup> The experimental evidence will be published in a separate paper.

light by oxidation. As Harvey found (6, p. 328), the writer also found that *Cypridina* luciférine can not be oxidized with light production by such oxidizing agents as neutral  $\text{H}_2\text{O}_2$ ,  $\text{PbO}_2$ ,  $\text{BaO}_2$ ,  $\text{KMnO}_4$  and  $\text{K}_2\text{Cr}_2\text{O}_7$ , although Dubois found just the contrary in *Pholas* luciférin. It is possible, however, that the conditions in *Pholas daetylus* may be radically different from those found in *Cypridina*. The writer has, therefore, no intention to make any claim against Dubois' conclusion.

#### THE DISTRIBUTION OF LUCIFÉRINE AND LUCIFÉRASE

According to Dubois, *Pholas* luciférine exists only in the luminous organs of the animal while the luciférase occurs throughout its body and also in many other non-luminous animals. On the contrary, Harvey finds that *Cypridina* photogenin (Dubois' luciférase) is only in the luminous organs of the organism, while *Cypridina* photophelein (Dubois' luciférine) "is found in many other non-luminous animals and in the non-luminous parts of *Cypridina hilgendorffii*" (6, p. 323).

The writer removed the shells of ten dried *Cypridina*s and cut them into anterior parts with the maxillary gland cells and posterior parts with no gland cells. Special precaution was taken not to mix one set with the other. The water extract of these posterior parts was mixed with a water extract of dried crushed *Cypridina*s in which the luciférase was left. This operation was, of course, performed in a dark room. If the luciférine (Harvey's photophelein) existed in these posterior parts, as Harvey claims, the mixture of these two extracts just mentioned should produce light. But this was not the case. The anterior halves with the maxillary gland, however, gave a brilliant light when moistened. On the other hand, the water extract of these posterior parts was mixed with the boiling water extract of dried crushed *Cypridina*s in which the luciférine was left. If the luciférase (Harvey's photogenin) is present in the posterior parts of the animal, the mixture of these two extracts should produce light. This was not, however, the case. Each of these two series of experiments was repeated several times with no exception. The writer is forced, therefore, to conclude that neither luciférine nor luciférase occurs in the posterior part of dried *Cypridina*. In other words, the luciférine and luciférase of *Cypridina* are found in the maxillary gland cells of the animal but in no other part.

There is a possibility, however, that either luciférine or luciférase may exist in other parts of living *Cypridina* beside the maxillary gland,

even though they occur only in the maxillary gland of the dried animal. In other words, either of these substances might be destroyed by drying, although existing in the living. The fact that both the luciférine and luciférase in the maxillary gland cells of the animal are dried without impairment hardly favors this idea. But the writer repeated Harvey's experiments. One living Cypridina at a time was removed from sea water by means of cloth fixed on wire and was placed on blotting-paper to remove the water adhering to the shell. Of course it was still alive after this treatment. It was then quickly cut by sharp scissors into the anterior and posterior parts. Five of these posterior parts with no maxillary gland cells were mixed with the water extract of these anterior parts with the gland cells or with the water extract of dried crushed Cypridinas. If the luciférine (Harvey's photophelein) were present in the posterior part of the animal, as Harvey claims, the mixture of these two should give light, as the luciférase (Harvey's photogenin) is left in the water extract of these anterior parts or dried Cypridinas allowed to stand. The results were very irregular. Sometimes no light was produced and sometimes one or two of the posterior parts showed dimly lighted points, though they lasted for only a short time. On the other hand, five posterior parts of living Cypridinas were mixed with the boiling water extract of the anterior parts of the living or of the dried crushed Cypridinas. If the luciférase exists in the posterior part of the animal, the mixture of these two should give light, since the luciférine was left in the boiling water extract. The results were again irregular just as those of the other series described above.

If, therefore, one judged from the positive results of these two series of experiments, it might be said that both luciférine and luciférase occur in the posterior part of the animal where no maxillary gland cells are located. On the other hand, if judged from the negative results it is quite logical to say that neither luciférine nor luciférase is found in the posterior part of the animal. The latter alternative seems the truth, because the writer believes that the results ascribed to be "positive" are nothing but false results derived from the rough method of preparing the material for experiment. The difficulty of manipulating the living Cypridina should be taken into serious consideration. Harvey's contention expressed in such statements as, "By a careful quick scissors cut, the head end of Cypridina containing the luminous gland can be separated from the posterior half without any contamina-



tion of the latter with luminous secretion," (6, p. 325), is not beyond criticism.

As already stated, Cypridinas give off the luminous secretion, if they are removed from sea water and are touched with any object, scissors, for instance. It is, therefore, quite possible that the secretion may adhere to the posterior part of the animal. If so, any "positive" results would be deceptive. Unless this objection in preparation of the experimental material is completely wiped out, any positive results are of no value. Even Harvey's statements, if subjected to a careful examination, suggest the possibility of false results, thus: "We must try the experiment immediately because this substance disappears if the extract stands in presence of oxygen. In absence of oxygen or if the extract is boiled immediately (but not too long a time) the substance does not completely disappear even after one hour. There is, therefore, in the non-luminous parts, the substance photophelein which disappears even in the absence of photogenin (from luminous gland) unless the solution be boiled or oxygen excluded" (6, p. 325).

This quick disappearance of photophelein in the absence of photogenin seems to the writer to mean an adherence of a very small amount of the former to the posterior part of the animal.

Furthermore the same "positive" results could sometimes be obtained even in the mixture of the posterior parts with no maxillary gland cells and distilled water. That is to say, the posterior parts of the animal carefully prepared sometimes produced light when mixed with water. Not only that, but a careful examination in a dark room revealed the fact that some of the posterior parts of living Cypridinas dissected out in the same manner as above were already producing light before they were brought in contact with water or any solution—the luciférase solution, for example. These facts undoubtedly prove the adherence of luminous secretion to the posterior parts of the animals, most probably to the shells while handled in cutting.

With the same point in mind, the shells of dried Cypridinas carefully removed for this special purpose were tested with distilled water. Numerous bright small points of light appeared. This fact of course could not be explained unless the light-producing substances secreted are assumed to have adhered to the shells while the animals were handled and dried in the sun.

If Cypridina luciférine occurs in any other parts of the animal where the maxillary gland cells are not found, as Harvey claims, the existence



of two different kinds of gland cells, which is histologically proved, becomes totally meaningless. On the contrary, their existence constitutes very strong evidence of the separate and specific nature of their functions in the production of luminous secretion.

Harvey also claims that he found the photophelein (Dubois' luciférine) in many other non-luminous animals. He has given a list of many animals tested and he adds that "of these forms *Lepas* and *Chiton* gave the best light and of these two only *Lepas* gave light with dilute *Cypridina* photogenin" (6, p. 326). The writer, therefore, made cold and boiling water extracts of *Lepas* and *Chiton* and tested them with extracts of *Cypridina* luciférine and luciférase. In both cases the results were absolutely negative.

From the variety of evidence, both experimental and histological, considered above, the writer is forced to conclude that *Cypridina* luciférine and luciférase occur only in the maxillary gland cells of the organism but not in any other part or in other non-luminous animals. In other words *Cypridina* luciférine and luciférase are specific in the strict sense of the word.

In comparing the results regarding the distribution of light-producing substances in *Pholas*, *Cypridina* and other non-luminous animals obtained by Dubois, Harvey and the writer, the following table will assist in visualizing the contradictory points of each investigator's claim discussed above.

TABLE 1

*Distribution of the luciférine and luciférase in luminous and non-luminous animals*

| SPECIES OF ANIMAL                                     | DUBOIS     |           |            |           | HARVEY       |           |            |           | KANDA      |           |            |           |
|---|------------|-----------|------------|-----------|--------------|-----------|------------|-----------|------------|-----------|------------|-----------|
|   | Luciférine |           | Luciférase |           | Photophelein |           | Photogenin |           | Luciférine |           | Luciférase |           |
| Non-luminous animals                                  | —          |           | +          |           | +            |           | —          |           | —          |           | —          |           |
| Luminous animals                                      | In gland   | Out gland | In gland   | Out gland | In gland     | Out gland | In gland   | Out gland | In gland   | Out gland | In gland   | Out gland |
| <i>Pyrophorus noctilucus</i> and <i>pholadactylus</i> | +          | —         | +          | +         |              |           |            |           |            |           |            |           |
| <i>Cypridina hilgendorffii</i>                        |            |           |            |           | +            | +         | +          | —         | +          | —         | +          | —         |

## THE EFFECT OF PRESERVATIVES ON LUCIFÉRINE AND LUCIFÉRASE

As already stated, the writer tested the effect of pure ether, alcohol and chloroform as preservatives of dried Cypridinas. They were preserved in small bottles. Dried whole Cypridinas and dried crushed ones were separately preserved in each substance. From time to time they were tested with distilled water to see whether they would produce light or not. The strength of light produced by them was compared with that of freshly collected and dried Cypridinas as control. The following table will show the results tested after eight months. Of course the preserved animals with the luciférine and luciférase never emit light in these narcotics. The reason is that the luciférine and luciférase are insoluble in the chemicals. They are therefore of a non-lipoid nature. Solubility in water and light production go in union.

TABLE 2

*Effect of preservatives on the luciférine and luciférase of Cypridina hilgendorfi*

| TIME              | FRESH CON-<br>TROL | PRESERVED<br>CONTROL | ETHER |         | ALCOHOL |         | CHLOROFORM |         |
|-------------------|--------------------|----------------------|-------|---------|---------|---------|------------|---------|
|                   |                    |                      | Whole | Crushed | Whole   | Crushed | Whole      | Crushed |
| Eight months..... | 8*                 | 7                    | 6     | 5       | 4       | 3       | 2          | 1       |

\* Figures indicate degree of lumination.

The figures in the table indicate the degrees of brightness compared with that of control. The figure "1," for example, means the lowest of all, although it is quite bright. It will be noticed that the brightness of the light produced by dried whole Cypridinas preserved in a desiccator with  $\text{CaCl}_2$  for eight months is not very different from that of freshly dried crushed ones used for control.

The question arises: if the luciférine and luciférase are insoluble in the preservatives mentioned above, what causes the gradual loss of light-producing power? This is hard to answer. But a few possible explanations may be mentioned. In the first place the preservatives used may not be pure, because they were not purified or redistilled for this purpose. As is well known, the so-called absolute alcohol on the market is about 99.5 per cent at best. Even a little water contained in alcohol might be a cause of slow destruction of the light-producing substances. The same may be said in the case of ether. In

the case of chloroform the material did not sink for a long time due to the former's high specific gravity and perhaps the light-producing substances might be impaired by the moisture or oxygen of the air. The writer is therefore convinced that if extra-pure chemicals are used as preservatives and if the Cypridinas are thoroughly dried, the material may be kept quite long. At any rate the writer concludes that the reason why *Cypridina luciférine* and *luciférase* never gave light when mixed with ether, alcohol or chloroform, is their insolubility in these chemicals.

As far as ether is concerned, this conclusion accords with that of Harvey. He says: "Dried crushed Cypridinas may be extracted with six changes of ether during the course of two days without impairing in the least their power to produce light when again moistened. The luminous substance is therefore of a non-lipoid, ether-insoluble nature, as might be expected from the fact that it is extruded from the animal as a clear water-soluble, non-fluorescent secretion" (6, p. 321). He therefore distinctly recognizes the fact of ether-insolubility of the luminous substances, and also the fact of indestructibility of the substances in ether. In other words, he recognizes the fact that no light is produced when both the luminous substances, *luciférine* and *luciférase*, are present together in ether. Strangely enough, however, the same investigator states "It (*Cypridina luciférase*) will also give light if mixed with many pure substances as chloroform, ether, benzol, thymol, saponin, oleic acid, atropin, NaCl and others. Since most of the above substances could not possibly be oxidized by the *luciférase*, I conclude that they cause in some way the giving out of light in what Dubois terms *luciférase*" (6, p. 323).

Judging from these statements Harvey seems to identify the action of many pure chemical substances so diverse in nature with that of *luciférine*. If so, the writer cannot quite understand why he uses the special word "photophelein" (or Dubois' *luciférine*) to indicate one of two light-producing substances which acts in the same way toward the *luciférase* (Harvey's photogenin) as chloroform, ether, benzol, thymol, saponin, atropin, pilocarpin, hydrochinon, pyrocatechin, chloral hydrate, butyl alcohol, oleic acid, ortol, aesculin, dextrine, NaCl,  $\text{MgSO}_4$ ,  $(\text{NH}_4)_2\text{SO}_4$  or  $\text{K}_4\text{Fe}(\text{CN})_6$ , or is omnipresent "throughout the body of *Cypridina hilgendorffii*" and in "many non-luminous animals." Of course Harvey states, "It is hardly worth inquiring into the nature of the substances in each particular extract which may for convenience be collectively spoken of as photophelein, since

I have found a great many simple bodies which, mixed with concentrated photogenin in powder or crystal form, give rise to a bright light" (6, p. 327). If so, the substance "photophelein," which Harvey thinks he has found in the posterior part of *Cypridina hilgendorffii* and in many other marine animals, might be NaCl or  $MgSO_4$  which are present in sea water in a large amount.

The writer tested *Cypridina* luciférase solution mixed with pure ether, alcohol, chloroform, NaCl and  $MgSO_2$  in various ways to see whether it gives light or not. The results obtained were absolutely negative. Because, as already stated, there is no possibility of light production in the mixture of the luciférase solution and pure ether, alcohol and chloroform, since no light is produced when the luciférine and luciférase are present together in these chemicals. For this reason Harvey's conclusion that the "luciférase is the source of the light and the luciférine. . . . is something which causes the luciférase to emit light" is not tenable at all.

Although his statements do not make clear how he conducted his tests, Harvey gives two tables in which "the effect of saturation of solutions (one *Cypridina* to 25 cc.) of photophelein and photogenin with the four substances," that is, chloroform, ether, benzol and thymol, is summarized (6, p. 331). In the first place, however, we must consider from purely physico-chemical viewpoints the properties of these four substances. That is to say, to what extent these substances could be "saturated" with the luciférine and luciférase solutions. Ether is soluble to the extent of 1 part in 12 of water, chloroform to the extent of 0.712 part in 100 parts of water at 17.4°C., benzene to the extent of 0.082 part in 100 parts of water at 22°C. and thymol "is only very sparingly soluble in water." It seemed to the writer to be extremely doubtful that any one could get real results which are to be considered as the effect of saturation of solutions of the luciférine and luciférase with these four chemicals, though Harvey claims that he did.

The writer found that dried crushed *Cypridina*s produced a brilliant light when they were placed in the saturated water-solutions of ether, chloroform and benzene. No difference of brightness between the light of the animals in the solutions and that of the control in distilled water was perceptible. In other words, the solubility of the luminous substances was not affected by the small amount of ether, chloroform and benzene contained in the saturated solutions. Since neither luciférine nor luciférase when they are present together is destroyed



even by pure ether, chloroform and benzene, as has already been shown (except benzene), it is no wonder that such a small amount of ether, chloroform and benzene as is in the saturated water-solutions of these substances is not detrimental to either luciférine or luciférase. Harvey claims, however, "ether is especially destructive to the photophelein" (6, p. 331). It was not ether, in the writer's opinion, that was especially destructive to the luciférine in Harvey's experiments but it was water. As repeatedly pointed out, even a mere trace of water is destructive to both the luciférine and luciférase.

On the other hand, the writer also tested ether and benzene which had dissolved water to the full extent, that is, to the extent of 2.25 cc. in 100 parts of ether and to the extent of 0.211 cc. in 100 parts of benzene. The results were all negative, that is to say, dried crushed Cypridinas produce no light in ether and benzene thus treated.

It may be worth mentioning that the writer found that living Cypridinas give light when put in pure ether, alcohol and chloroform. This puzzle is readily explained by the following consideration: The living Cypridinas carry sea water inside their shells and secrete the luminous substances when they are placed in the chemical substances: and the secretion meets the sea water carried by themselves. The appearance of light is the result. Neither alcohol nor chloroform plays any rôle in this process.

#### PROTEIN TESTS ON LUCIFÉRINE AND LUCIFÉRASE

Harvey states that "despite the fact that the light from the natural secretion of Cypridina is very bright, a sample of the secretion, collected by shaking many Cypridinas in a small volume of sea water and filtering, responds to none of the common biochemical tests. . . . that both Cypridina luciférine and luciférase solutions are positive for proteins. . . ." (6, p. 322). The writer, however, found that both Cypridina luciférine and luciférase solutions are positive to the biuret, xanthoproteic and other tests. The experiments are in progress and will be given in a separate paper.

#### THE EFFECT OF TEMPERATURE ON LUCIFÉRINE AND LUCIFÉRASE

Harvey found that "Cypridinas dried over  $\text{CaCl}_2$ , ground, and the powder suspended in sea water give a beautiful light which disappears when heated to  $56^\circ$ , but returns on cooling. If heated to  $65^\circ$  and cooled, the light also returns, but does not return if heated to  $70^\circ$  and

then cooled" (6, p. 329). He also found that "the luminous material of *Cypridina*. . . . is unaffected by cold and will glow brilliantly at 0°C." The writer tested the effect of temperature on light production of the animals in a different way from that of Harvey and found practically the same results as the latter. The method will be stated in brief.

The writer heated 40 cc. of distilled water to various temperatures and put twenty dried crushed *Cypridinas* in it for about thirty seconds (or sometimes for one minute). The water with the animals was then poured into 300 cc. of distilled water which was at about 25°C. In this way the effect of different temperatures upon the light-producing substances was studied, as shown in the accompanying table (table 3).

TABLE 3

*Effect of temperature on the luciferine and luciferase of Cypridina hilgendorffi*

| TEMPERATURE | HARVEY |        | KANDA  |        |
|-------------|--------|--------|--------|--------|
|             | Heated | Cooled | Heated | Cooled |
| °C.         |        |        |        |        |
| 70          | —      | —      | —      | —      |
| 69          |        |        | —      | —      |
| 67          |        |        | —      | —      |
| 65          | —      | +      | —      | +      |
| 60          |        |        | —      | +      |
| 59          |        |        | +      | +      |
| 56          | —      | +      | +      | +      |
| 0           |        | +      |        | +      |

#### SUMMARY AND CONCLUSIONS

1. The animal used for these experiments was *Cypridina hilgendorffi*.
2. The animal is generally negatively heliotropic, but some individuals of the species are positive to light.
3. The living animal is not good material for experimental work. It is better to use the animal thoroughly dried in sunlight.
4. The dried animal may be preserved in a desiccator with  $\text{CaCl}_2$  for over eight months apparently without impairing in the least its power to produce light when again moistened. It may also be preserved with some deterioration in pure ether, alcohol or chloroform for over seven months.
5. Ether is the best preservative.

6. The maxillary gland cells of the dried animal are dark red. The red substances produce light when dissolved in water.

7. Water is essential for the production of light.

8. Two substances are concerned in the production of light: the one is destroyed by boiling and the other not. As these two substances show practically the same reaction of luciférine-luciférase as those in *Pholas dactylus* studied by Dubois, the writer uses Dubois' words, "luciférine" for the thermostable substance and "luciférase" for the thermolabile substance.

9. Harvey's theory of photophelein-photogenin does not fit the author's experimental results.

10. The luciférine and luciférase of *Cypridina hilgenforii* are found only in the maxillary gland cells of the animal. Neither luciférine nor luciférase is found in the non-luminous animals.

11. The luciférine does not produce light when mixed with  $H_2O_2$ ,  $KMnO_4$ ,  $PbO_2$  and so forth.

12. The dried animal does not produce light in pure ether, alcohol and chloroform. The luciférine and luciférase are not soluble in these chemical substances. They are therefore of non-lipoid nature.

13. The living animal may produce light in pure ether, alcohol and chloroform. These chemical substances, however, play no rôle in such production of light. It is due to sea water adhering to the animal.

14. The dried crushed animal gives light in a saturated water solution of ether. This is due to the water, not to the ether.

15. The luciférine and luciférase solutions give the color tests for proteins.

16. The effect of temperature is given in table 3.

The writer concludes that Harvey's new theory and new words are not tenable. The so-called photogenin (Dubois' luciférase) is not the source of the light but may be an enzyme. The photophelein (Dubois' luciférine) is the source of the light.

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## PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE

### II. THE PRODUCTION OF LIGHT BY *CYPRIDINA HILGENDORFII* IS NOT AN OXIDATION<sup>1</sup>

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#### INTRODUCTORY

That the production of light and oxidation are intimately related to each other is a very general phenomenon judged from common sense and considered from the viewpoints of physics and chemistry. It is, therefore, no wonder that we find in the literature on the subject of the relation of oxygen to light production in living organisms, the statement that the production of light by bacteria, *Noctiluca*, *Pholus*, fire-flies and others, is an oxidation (4, p. 353-358).

Harvey (2, p. 321) who has studied the action of oxygen on the production of light by *Cypridina hilgendorffii*, concludes:

Oxygen is necessary for light production as may be seen by placing the crushed animals in an hydrogen atmosphere, or by bubbling hydrogen through a glowing extract of the animals. The light never completely disappears even after a long time, but remains dim so that very little oxygen (as no special precautions were taken to remove the last traces of oxygen from the hydrogen, prepared in a Kipp generator) is sufficient to give light. Upon readmitting oxygen, however, a brilliant glow results. Every other species of animal investigated likewise requires oxygen for phosphorescence.

The writer also imagined that "oxygen and water are necessary for the production of light by *Cypridina hilgendorffii*" and fancied that luciferase "is an oxidizing enzyme" (3, pp. 321 and 448). But the results of his recent work, which was carried out under more careful experimental conditions, have contradicted his expectation. That is to say, he has found that the light production in *Cypridina* is by no means a phe-

<sup>1</sup> This paper was published in the Japanese Journal of Zoölogy, xxxi.

nomenon of oxidation. Oxygen is not necessary for the production of light by this animal as will be shown in this paper.

The following experiments were conducted in the Science Department of the Kyushu Imperial University, Fukuoka, Japan. The writer expresses his appreciation of the interest and suggestions of Dr. Tsuneya Marusawa, Professor of Physical Chemistry in the University, throughout the course of the work. To Prof. Ayao Kuwaki and all the members of the department, the writer acknowledges his gratitude for the privileges of the laboratory and their interest in the work. The work was supported by the private contribution of Mr. Jihachi Hamano.

#### MATERIAL

*Cypridina hilgendorffii* were thoroughly dried in the direct sunlight. The animals were then crushed and the shell and body separated by means of a sieve. The body material containing the maxillary glands was extracted with several changes of ether during the course of a few days "without impairing in the least their power to produce light when again moistened." For convenience's sake, the bodies thus prepared are hereafter called the "experimental material" or simply "material."

#### DESCRIPTION OF APPARATUS

Believing in Harvey's statements, the writer first tried "by bubbling hydrogen through a glowing extract" or a glowing mixture of distilled water and *Cypridinas* to see whether the light disappears or not. Hydrogen gas was prepared in a Kipp generator. The method was, however, so crude that he could not obtain decisive results. At the suggestion and also under the direction of Doctor Marusawa, therefore, the writer conducted all his experiments with the following apparatus. The arrangement of the apparatus as shown in figure 1 is typical although it was variously modified for the different gases employed.

The apparatus consists of two wings, right and left. Each wing has an experiment bottle, *E* or *E*<sub>1</sub>, of a capacity about 60 cc. The bottle is fitted with a tight rubber stopper in which three glass tubes, *A*, *B* and *C*, or *A*<sub>1</sub>, *B*<sub>1</sub> and *C*<sub>1</sub>, with one stop-cock for each, are inserted with the arm bent at 90 degrees. In the case of the bottle *E*, the glass tube *B* with a stop-cock *b* is connected to a T-shaped glass tube, *D*, with a stop-cock, *d*, by means of rubber tube. The second arm of the glass tube *D* is connected to the flask *W* by means of glass and rubber tubes,

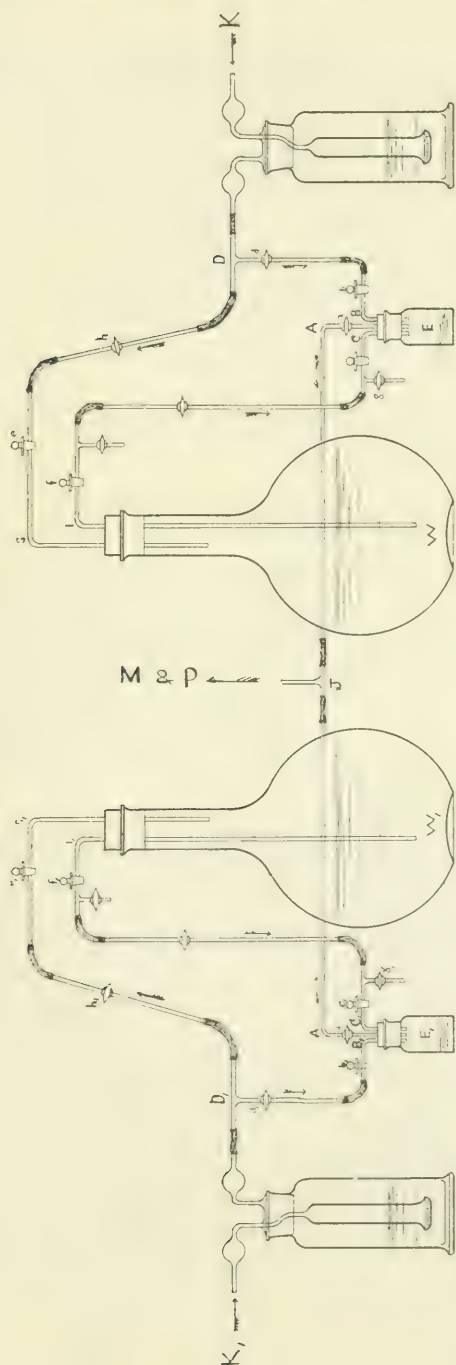


Fig. 1

and the third leads to the Kipp generator, *K*, through the gas wash bottle. The glass tube *C*, which has a stop-cock *c*, and is T-shaped, is connected to the flask *W* by means of glass and rubber tubes, and a third arm has a stop-cock *g* by means of which water from the flask *W* is caused to flow. The glass tube *A* with a stop-cock *a* is connected to a T-shaped glass tube *J*, the second arm of which leads to a Gaede oil pump, *P*, through a manometer, *M*, and a safety bottle and the third to the glass tube *A*<sub>1</sub> of the experiment bottle *E*<sub>1</sub> at the left wing of the apparatus. The arrangement of the bottle *E*<sub>1</sub> is exactly similar to that of the bottle *E*, though sometimes a gas holder is used, depending on the gas under investigation. So the Kipp's generator is not in permanent use.

The apparatus was placed in a dark room, to facilitate the observation of the production of light.

#### THE METHOD OF EXPERIMENT

As the chief purpose of the following experiments was to determine whether the production of light by *Cypridina hilgendorffii* is an oxidation or not, a hydrogen experiment was always conducted together with any other gas experiment as a control, besides a control for which air was used. Special care was, of course, exercised when an oxygen experiment was performed. The principle of the method is that the production of light by the material can be observed in the water free from any gases or in any pure gas.

In the first place, therefore, the distilled water to be used should be thoroughly heated by boiling for a few hours. While the water is still boiling the flask *W* or *W*<sub>1</sub> is fitted with a tight rubber stopper which carries two glass tubes, long *l* and short *s*, tightly fitted. Each of these two tubes has a stop-cock. Steam comes out from the outer ends of these two tubes within a few minutes, since the water is still boiling. These tubes are then closed by the stop-cocks *e* and *f*. The glass tube *s* or *s*<sub>1</sub>, as the case may be, is now ready to be connected to one arm of the glass tube *D* or *D*<sub>1</sub> by means of glass and rubber tubes.

One of the most important procedures in this method is to have any desired gas for experiment prepared in the Kipp's generator or the gas holder connected with the gas wash bottles and with the glass tube *D* or *D*<sub>1</sub> before the glass tube *s* or *s*<sub>1</sub> of the flask *W* or *W*<sub>1</sub> is to be connected to one arm of the glass tube *D* or *D*<sub>1</sub>. In other words, any desired gas should be ready to pass through the gas washers and the glass tube *D*



or  $D_1$  before the glass tube  $s$  or  $s_1$  of the flask  $W$  or  $W_1$  is connected to one arm of the latter, and the stop-cock of the glass tube  $s$  or  $s_1$  is opened. Because the water and also the space in the flask  $W$  or  $W_1$  should be filled up with the desired gas, while the water becomes cooler and cooler. Special care should, of course, be taken that any air in any glass and rubber tubes should be excluded by all possible means, whenever any connection is made. Before the glass tube  $s$  or  $s_1$  of the flask  $W$  or  $W_1$  is, therefore, to be connected to the tube  $D$  or  $D_1$ , the washers should be evacuated and then filled with the gas to be used up to the stop-cock  $h$  or  $h_1$ .

All being thus prepared, the tube  $s$  or  $s_1$  is connected to the tube  $D$  or  $D_1$  without any unnecessary delay, while the water is still boiling and then the stop-cocks  $e$  or  $e_1$ ,  $h$  or  $h_1$  and  $d$  or  $d_1$ , in order, are opened. Hot steam and gas come out from the end of the arm, because the water is still boiling and the gas is slowly generating. All air is thus driven out from all spaces and the stop-cock  $d$  or  $d_1$  is closed. At the same time the flask  $W$  or  $W_1$  is removed from the flame. Everything thus arranged, the water and space in the flask  $W$  or  $W_1$  are gradually filled up by the gas until the temperature of the water becomes equal to that of the room. When the temperature equilibrium of the inside and outside of the flask  $W$  or  $W_1$  has been established, the glass tube  $l$  or  $l_1$  is pushed down into the water, and is now ready for its connection to one arm of the glass tube  $C$  or  $C_1$  of the experiment bottle  $E$  or  $E_1$ .

Four bottles, two for the experiment and two for the control, of similar capacity and shape, are cleaned and dried thoroughly. In each of these bottles 0.2 gram of the experimental material is placed. The experiment bottles are then fitted with tight rubber stoppers each of which carries three glass tubes as previously described. One arm of the glass tube  $C$  or  $C_1$  which is T-shaped is now connected to the glass tube  $l$  or  $l_1$  of the flask  $W$  or  $W_1$ . And then the gas-saturated water is introduced up to the stop-cock  $c$  or  $c_1$ , by the management of one arm of the tube  $C$  or  $C_1$ . The bottle  $E$  or  $E_1$  is fixed on an iron stand. Now the connections of the tube  $B$  or  $B_1$  through the tube  $A$  or  $A_1$  to the T-shaped tube  $J$  and the third arm of the last to the pump,  $P$ , through the manometer,  $M$ , and also a safety bottle, are made by means of heavy-walled rubber tubes. All arrangements thus made, the apparatus is now ready for experiment.<sup>2</sup>

In the first place, the stop-cocks  $a_1$  and  $b_1$  are opened and at the same time the pump is started to evacuate all air in the bottle  $E_1$  and the

<sup>2</sup> It is convenient to begin any experiment from the left wing first.

rest of the apparatus. A complete evacuation of the air, however, is impossible by one operation. In order to complete the evacuation of the air, therefore, it is necessary to fill up all the spaces again with the gas desired, which has been prepared for the experiment. The stop-cock  $d_1$  is now opened, and the gas fills all the spaces. After this filling is complete, the stop-cock  $d_1$  is closed and the pump is again started. This same procedure is repeated ten times, though five times are sufficient. After the last filling with the gas, the stop-cocks  $a_1$  and  $b_1$  are closed. The last procedure is simply to pour a necessary amount of water from the flask  $W_1$  into the bottle  $E_1$  for an observation of the production of light by the material in the bottle. But this should not be done until the bottle  $E$  has been prepared in the same way.

To do this the pump  $P$  is again started to evacuate the gas in all tubes and spaces from the pump and up to the points of the stop-cocks  $a$  and  $a_1$ . After this evacuation, the stop-cocks  $a$ ,  $b$  and  $d$  are opened. In so doing, another gas is introduced to fill the bottle  $E$  and the rest of the apparatus. After this filling the stop-cock  $d$  is closed and the pump is again started. After this evacuation, the stop-cock  $d$  is opened and fresh gas is permitted to enter. This procedure is repeated ten times as before. After the gas has filled bottle  $E$  and the rest of the apparatus, all the stop-cocks,  $a$ ,  $b$  and  $d$ , are closed. The bottle  $E$  is now also ready to receive water.

Now the stop-cock  $c$  is opened and about 20 cc. of the water are poured from the flask  $W$  into the bottle  $E$ . At the same time, about 20 cc. of the ordinary distilled water are also poured in one of the control bottles which is fitted with a tight stopper. The time should be recorded because it is a very important factor in the production of light. Then the stop-cock  $c_1$  is opened and about 20 cc. of the water are poured from the flask  $W_1$  in the bottle  $E_1$ . At the same time the second control is prepared with water. These procedures take about 2 to 3 minutes. Then the bottles  $E$  and  $E_1$  with the tubes,  $A$ ,  $B$  and  $C$ , and  $A_1$ ,  $B_1$  and  $C_1$  are freed from all the connections of the rest of the apparatus. At the same time the room is to be darkened. The production of light by the material in the bottles  $E$  and  $E_1$  is thus to be observed together. As already stated, special care should be taken that the controls are to be set up at the same time when the water is poured in the bottles  $E$  and  $E_1$ , since time is a very important factor for the observation of the intensity of the light produced by the material. At the time of pouring water, therefore, at least three persons are to be ready for the work, to save time.

The writer is convinced that the method and apparatus described above are satisfactory to determine the effect of any particular gas on the material.

#### THE PREPARATION AND USE OF GASES

The gases used for these experiments were hydrogen, oxygen, nitrogen, carbon dioxide and carbon monoxide. Of these gases, hydrogen, nitrogen, carbon dioxide and carbon monoxide were prepared in the laboratory, and oxygen in bomb was used. The methods of preparing these four gases were those in common use. The writer thinks, however, that brief statements about the methods of preparing them may not be out of place, because all the procedures carried out by the writer need to be open for free discussion.

1. The preparation of hydrogen: Hydrogen was prepared with a Kipp's apparatus in which zinc and about 50 per cent  $\text{H}_2\text{SO}_4$  were placed. The liberated gas was washed by passing it through four wash bottles. Distilled water was placed in the first, and saturated  $\text{KMnO}_4$  solution in the second, a solution of 30 grams of  $\text{KOH} + 10$  grams of  $\text{C}_6\text{H}_3(\text{OH})_3$  in 100 cc. of distilled water in the third, and concentrated  $\text{H}_2\text{SO}_4$  in the last. As hydrogen was always used as a control in order to compare the action of this gas with that of any other gas, this Kipp's apparatus was always fixed in the right wing. The last wash bottle of this apparatus, therefore, was always connected to the tube, *D*. The solutions in the wash bottles were renewed from time to time.

2. The preparation of carbon dioxide: Carbon dioxide was also prepared with a Kipp's generator in which pieces of marble and about 15 per cent  $\text{HCl}$  were placed. The gas when liberated was washed by passing it through three wash bottles. Distilled water was placed in the first and second and concentrated  $\text{H}_2\text{SO}_4$  in the third. This last wash bottle was connected to the tube, *D*<sub>1</sub>, when used for experiment.

3. The preparation of nitrogen: In the first place, 200 grams of  $\text{NaNO}_2$ , 300 grams of  $(\text{NH}_4)_2\text{SO}_4$  and 200 grams of  $\text{K}_2\text{CrO}_4$  were barely dissolved in separate beakers. The solutions were placed together in a 5-liter flask and 1500 cc. of distilled water were added to it. A condenser was connected to the stopper of the flask in order to cool the nitrogen gas. To this condenser, a heavy Erlenmeyer flask as a safety bottle and two wash bottles were connected. In each of these bottles 30 cc. of concentrated  $\text{H}_2\text{SO}_4$ , 20 grams of  $\text{K}_2\text{Cr}_2\text{O}_7$  and 100 cc. of distilled water were placed.



The mixture contained in the flask was slowly heated on a low flame. When enough  $N_2$  gas was liberated, the last wash bottle was connected to a gas holder which was already prepared to receive the gas. Special care was taken to have no air in any space. When the gas in the gas holder was used for experiment, it was again washed by passing through the same wash bottles as mentioned above.

4. The preparation of carbon monoxide: In a liter distillation flask, 500 cc. of 80 per cent  $H_2SO_4$  were placed. In the rubber stopper of this flask, one long thermometer of  $200^\circ C$ . and one glass tube perforated on its sealed end of about 5' cm. with many small holes were inserted. The mercury part of the thermometer and the hole part of the tube were entirely dipped into the sulphuric acid of the flask. The outer end of the tube was bent about 90 degrees and a suitable rubber tube with a pinch-cock was connected to it. The other end of the rubber tube was then connected to a separating funnel in which some formic acid was placed. To the distillation flask, a heavy Erlenmeyer flask as a safety bottle, and two wash bottles, in each of which 200 cc. of 20 per cent NaOH were placed, were connected. The sulphuric acid of the flask was slowly heated on a low flame to about  $110^\circ C$ ., and the formic acid was added little by little. After enough gas was liberated, the gas was received in a gas holder.

When the carbon monoxide gas was used for experiment, it was again washed by passing through three gas wash bottles. Two hundred cubic centimeters of 20 per cent NaOH were placed in the first and second bottles, and 200 cc. of concentrated  $H_2SO_4$  in the third, which was connected to the tube,  $D_1$ .

#### EXPERIMENTAL

With the methods and apparatus described in the previous section, the writer conducted his experiments with every possible care and repeated each series of experiments four or five times, even though no exception was found in any trial. The results of these experiments are summarized in table 1.

The figures of the table show a relative superiority and inferiority of the intensity<sup>3</sup> of light produced by the material on which five independ-

<sup>3</sup> The observation of the intensity of light was made at the moment when the two experimental and two control bottles held together in both hands were given an equal shaking. A question may arise about the intensity of light, because the writer has not determined it by a quantitative method. According to the calcu-



ent gases and air were allowed to act. The figure "6," for example, means the highest degree of light intensity compared with all others. As repeatedly stated, the experiments on the action of hydrogen and of air were always made as controls of any other gases. But as the experiments on the other gases, carbon dioxide and carbon monoxide, for instance, were not carried out together, it was impossible to compare their light intensities at the same time. The time for observing the light intensity in each gas, however, was carefully recorded in comparison with that of light produced in hydrogen and in air. In this roundabout way, therefore, the action of each gas may be compared. Furthermore, it was enough if the actions of hydrogen and oxygen were

TABLE 1

*Comparative intensity of light produced by the material in various gas atmospheres and water*

| TIME OBSERVED IN<br>DARK ROOM | LIGHT INTENSITY OF THE MATERIAL OBSERVED AT A GIVEN TIME IN THE<br>ATMOSPHERES AND WATER OF THE FOLLOWING |                |    |                 |     |                |
|-------------------------------|---|----------------|----|-----------------|-----|----------------|
|                               | H <sub>2</sub> (Special<br>control)   | N <sub>2</sub> | CO | CO <sub>2</sub> | Air | O <sub>2</sub> |
| 1 m.                          | 6   | 6              | 6  | 6               | 6   | 6              |
| 3-4 m.                        | 6   | 6              | 6  | 5               | 4   | 4              |
| 5-8 m.                        | 6   | 6              | 5  | 5               | 3   | 3              |
| 12-15 m.                      | 6   | 5              | 4  | 4               | 3   | 2              |
| 20 m.                         | 6   | 5              | 4  | 4               | 2   | 1              |
| 9-10 h.                       | 5   | 4              | 3  | 3               | 1   | 0?             |
| 13 h.                         | 4   | 3              | 2  | 3?              | 1   |                |
| 160 h.                        | 1   | 1              | 0  | 0               | 1?  |                |
| 200 h.                        | 0?  | 0              |    |                 | 0   |                |

accurately compared, since the essential problem of these experiments was to determine whether the production of light by the animal was an oxidation or not. There was an unmistakable difference of the light intensity between the actions of oxygen and air. The difference of the intensity of light produced in the hydrogen and oxygen atmospheres, as well as in the air, was so astonishingly marked that no one could

lation of V. Henri et Larguier des Bancelles, however, the retina is very "sensitive to an amount of light energy as small as 5 times  $10^{-12}$  ergs. This is about three thousand times as sensitive as the most rapid photographic plate" (1, p. 512). If so, although there is no quantitative means to decide the weak or strong intensity of light observed by the retina, there is no indicator superior to the qualitative judgment of the retina. It is believed, therefore, that the decision made by the retina is most accurate.

question it. And the action of the nitrogen gas in comparison with the action of carbon dioxide or of carbon monoxide was also very distinct. Superiority or inferiority between the actions of CO and CO<sub>2</sub> may be questioned, though the writer felt that the former was a little superior to the latter.

It will be worth while to describe some other facts than those shown in table 1. In the water saturated with air and other gases, the color of light produced by the material is bluish white, a few hours after the treatment, while in the water saturated with hydrogen, *a*, it is decidedly blue. This blue color in the latter lasts almost as long as the light continues. In the cases of air and other gases the light after shaking takes about three or four seconds to return to the state before shaking, while *b*, in the case of hydrogen it takes about eight seconds. In a perfectly dark room it is observed that the strong light after shaking lasts quite long. As the time ratio of durability, 1:2, however, is not altered, no deviation is involved in this observation. Generally speaking, the "steady homogeneous glow" of light is to be observed, as Harvey pointed out. But *c*, the light in the hydrogen-saturated water is observed to be heterogeneous by the flowing and precipitating of extremely minute particles when shaken in a dark room. Harvey's claim of "complete proof of the truly soluble character of the light-producing substances" (2, p. 321) may not be true. The writer will try to make this point clear by using an ultramicroscope in the near future. In the resting state, *d*, the light in the case of hydrogen glows as if the solution glows itself but the glow of the individual points of the material is not so marked as it is in the cases of air and any other gases, while in the latter cases the solution is clear. And *e*, in the latter cases also the solution forms when shaken but not so much as in the former. In the cases of all gases, when the production of light is near to the end, the solution does not glow as a whole but only at the surface when shaken. This *f*, is markedly so in the case of hydrogen. But in the case of air, the solution glows as a whole till the end.

These are six characteristics of the effect of the hydrogen gas on the production of light by the material besides those shown in table 1. Whether these are of any significance regarding the light production or are simply to be overlooked as meaningless, can not be settled unless further facts are found.

After an experiment was over, a test was made with a lighted match to see whether the gas used was present or not. The results were always positive even after seventy-five days had passed. Each solution

was also tested with litmus paper to see whether it was alkaline or acid. In the cases of hydrogen, nitrogen, oxygen and air, the solution was found to be neutral, although the solution was slightly alkaline after about two hundred hours in the last case. In the case of carbon dioxide, the solution was always acid and was distinctly milky with white precipitation. In the case of carbon monoxide, the solution was also acid, though very faint; and the acidity seemed to increase a little after about two hundred hours. In the cases of oxygen, air and carbon monoxide, the solution and material became very black, while in the case of hydrogen and nitrogen the solution was clear and the material became reddish brown.

#### CONCLUSION AND DISCUSSION

That the less the quantity of oxygen contained in the experiment bottle the more intense is the light produced by the material after the first minute is quite obvious according to the results stated above. The light produced is more intense in the water saturated with air than in the water saturated with oxygen and is markedly more intense and durable in the water saturated with hydrogen or nitrogen than in the water saturated with oxygen or air. In other words, oxygen is not necessary for the production of light by the material. If so, there is no ground for the assumption that the production of light by the animal is an oxidation. If the production of light by the animal is due to an oxidation, as Harvey claims, the more intense light should be produced by the greater concentration of oxygen. This is not the case, and the writer therefore concludes that the production of light by the animal is not an oxidation.

A question arises whether the production of light by the material is a reduction or simply an hydrolysis. In expectation of getting some light on this question, the writer made experiments with carbon monoxide as a reducing substance. But the results were not so marked as expected. That is to say, the results were not as good as those in the case of hydrogen. If so, is this not a reduction? This question is not settled as yet. Carbon monoxide is a "poison gas." It may, therefore, act as a poison just as it acts on the hemoglobin of the blood. At any rate, it may be necessary to take into such biological consideration some factors besides the action of carbon monoxide purely looked upon from the viewpoint of chemistry. If so, it is no wonder that the action of carbon monoxide on the production of light by the material is inferior

to that of hydrogen or nitrogen. Whatever the exact interpretation of the facts may be, no decisive conclusion can be made unless further facts are found.

As to the action of carbon dioxide, there is the same question as in the case of carbon monoxide. If the light production of the material is not an oxidation, the intensity of light produced should be the same in carbon dioxide as in hydrogen or nitrogen. But this is not the case. However, if the fact is considered that carbon dioxide is dissolved in water and carbonic acid is formed, the riddle may be readily solved. Because it is a well-known fact that acid is injurious to organisms while alkali is not.

Furthermore, there are other points in table 1 which require explanation. In the first place, the light-producing substances in the water saturated with oxygen disappear fastest in spite of the production of the poorest light. On the other hand, the production of light in the water saturated with hydrogen is most intense and most enduring. Such phenomena may be explained by the following assumptions. That the higher the oxygen tension is the faster the oxidation of any substance is obvious. Although the production of light in question is by no means an oxidation, the light-producing substances, especially the luciférine, may always be subjected to an oxidation and thus the disappearance of the substance is apparent. If it be true that the higher the oxygen tension is the more the light-producing substance or substances may be oxidized (though this oxidation of the substances has no bearing on the light production), it is no wonder that the light becomes weaker and disappears faster if the substance or substances become less and less by an oxidation. Such a consideration explains the facts that the light is weakest and lasts shortest in the water saturated with oxygen and on the other hand, that the light is strongest and lasts longest in the water saturated with hydrogen with no oxygen.

If so, the action of the water saturated with carbon monoxide, carbon dioxide and nitrogen might be the same as the action of the water saturated with hydrogen. But this is not the case. As to the action of the first two gases there may be possibly some physiological or biological factors involved, as already stated. So the mere fact that free oxygen is not present should not be looked upon as a complete explanation. Taking this as granted, then, how is it in the case of nitrogen? The difference in action of hydrogen and nitrogen is not very marked, but it is still easily distinguishable. It may be said, therefore, that the superiority of the action of hydrogen over any other gas is its own speci-



ficity. In brief, the writer should confess that he has not yet clear knowledge with which he can explain these various difficulties. He expects to make special efforts to gather all possible data based on experiments. These difficulties may be of such a nature that they may be explained when further facts are available.

#### SUMMARY

1. The intensity of light produced by the material is strongest and lasts longest in the water saturated with hydrogen.

2. The intensity of light produced by the material is weakest and lasts shortest in the water saturated with oxygen. Therefore the production of light by the material is not an oxidation.

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# THE PREPARATION OF ADENINE NUCLEOTIDE BY HYDROLYSIS OF YEAST NUCLEIC ACID WITH AMMONIA<sup>1</sup>

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When yeast nucleic acid is heated in an autoclave with ammonia for several hours at 145°–148°, it completely loses its phosphoric acid so that its four nucleotides are formed (1), (2).

But when yeast nucleic acid is similarly treated with ammonia at a lower temperature (105°–120°) it does not lose any of its phosphoric acid but decomposes into nucleotides (3).

After hydrolysis at the lower temperature, if one will treat the product alkaline as it is, with an equal volume of alcohol, a very useful separation occurs. The guanine complex is precipitated while the adenine complex remains in solution (3), so that the material can be separated sharply into two fractions. This is the analytical key to the separation of adenine nucleotide from guanine nucleotide and without it the results discussed below could not have been obtained. It is based upon the fact that alkaline salts of adenine nucleotide are quite soluble in 50 per cent alcohol while the alkaline salts of guanine nucleotide are practically insoluble (4).

From the *adenine fraction* Jones and Germann (3) prepared the free nucleotide by means of its lead salt and found that it contained a uracil group in addition to the adenine group. From the fact that precisely half of its phosphoric acid is "easily split" and half is "firmly bound" Jones and Read (5) concluded that the substance contains its adenine and uracil groups in equivalent quantities, and is therefore adenine-uracil di-nucleotide. This conclusion appeared to be much strengthened by the following experiment which Jones and Read did not publish. An attempt was made to obtain uracil nucleotide after destroying the

<sup>1</sup> While this article was in press, the contribution of Levene appeared. Journ. Biol. Chem., 1919, xl, 415.

adenine nucleotide with boiling mineral acid. One hundred grams of the supposed di-nucleotide were used for this purpose but 98 grams were destroyed leaving only 2 grams of undecomposed nucleotide material. This was found to have all the properties of adenine-uracil di-nucleotide including the formation of a crystalline brucine salt having the required chemical composition. Thus it appeared that the adenine- and uracil-nucleotides were not only present in equal quantities, but were destroyed in equal quantities.

Jones and Read (6) prepared from the di-nucleotide a crystalline brucine salt which had sharply the composition required for the brucine salt of adenine-uracil di-nucleotide.<sup>2</sup>

Levene (7) prepared this nucleotide by the method of Jones and Germann and converted it into its brucine salt as described by Jones and Read. He states that the brucine salt which he obtained possessed the analytical values required for adenine-uracil di-nucleotide, but found that these values are disturbed when the substance is repeatedly recrystallized from 35 per cent alcohol. After nine successive crystallizations, the surviving brucine salt had a chemical composition very close to that required for the brucine salt of uracil nucleotide. From this brucine salt a crystalline barium salt was prepared and found identical with the barium salt of uracil nucleotide that Levene (8) had already prepared from another source.

Levene's results appeared very convincing to us in so far as they went to show that the supposed adenine-uracil di-nucleotide is in reality a mechanical mixture of its component mono-nucleotides, and his results also suggested to us the possibility of preparing adenine nucleotide from the mixture; for Jones and Kennedy (9) had prepared adenine nucleotide and had found its properties such as should make its isolation from almost any mixture a comparatively easy matter. By the application of their method to this problem, we have isolated pure crystalline adenine nucleotide identical with the substance described by Jones and Kennedy.

Thirty-eight grams of nucleotide were dissolved in 152 cc. of hot water and treated with a solution of 95 grams of brucine in the smallest possible amount of alcohol. On cooling, the material stiffened to a paste of crystals which was filtered on a Buchner funnel and crystallized from 35 per cent alcohol. Sixty grams of this brucine salt were treated

<sup>2</sup> This can be accurately determined because adenine nucleotide and uracil nucleotide differ from each other markedly in composition. The one contains five atoms of nitrogen; the other, only two.

with 6 liters of cold 35 per cent alcohol and after digestion with occasional agitation for 24 hours, the alcoholic extract was filtered from the undissolved residue on a Buchner funnel and allowed to evaporate for several days in open dishes at the room temperature. As the alcohol escaped, a crystalline brucine salt was deposited which was collected, allowed to dry in the air and used for the preparation of adenine nucleotide as described below (25 grams).

The undissolved residue from the alcoholic extraction was again extracted with 600 cc. of cold 35 per cent alcohol and the dissolved brucine salt was recovered after evaporation at the room temperature. This had very nearly the composition of the brucine salt of uracil nucleotide.

0.6136 required 12.04 cc. of standard  $\text{H}_2\text{SO}_4$  (1 cc. = 0.003642 N).

|        | REQUIRED FOR       |                   | FOUND |
|--------|--------------------|-------------------|-------|
|        | Adenine nucleotide | Uracil nucleotide |       |
| N..... | 10.00              | 6.79              | 7.15  |

Thus the original extraction with 6 liters of cold alcohol had almost completely removed the brucine salt of adenine nucleotide, but of course the extract contained also a considerable amount of the brucine salt of uracil nucleotide.

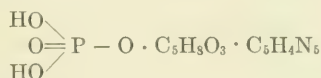
Twenty-five grams of the brucine salt mixture (from the 6 L of 35 per cent alcohol) were suspended in 800 cc. of boiling water and treated with a little ammonia which takes it promptly into solution. As the solution cooled, it was made and kept faintly but distinctly alkaline with ammonia. In this way the brucine is thrown down in easily filterable crystalline needles. The material was kept overnight in the ice chest, filtered from the brucine with a pump and shaken in a separating funnel with several successive portions of chloroform to remove the last traces of brucine. The solution was then evaporated<sup>3</sup> at 45° under diminished pressure to one-third of its volume and treated in the warm with a slight excess of lead acetate. The heavy granular lead compound was filtered on a Buchner and washed by grinding with hot water in a porcelain dish. Finally the material was made into a smooth suspension with warm water, decomposed in the warm with sulphuretted

<sup>3</sup> The neutralization of the ammonia with acetic acid as done by Jones and Kennedy should be omitted since the lead salt of adenine nucleotide is quite soluble in ammonium acetate.



hydrogen and the solution obtained after filtering off the lead sulphide was aspirated to remove the excess of sulphuretted hydrogen. On standing over night this solution deposited snow white adenine nucleotide in characteristic needles. The mother liquor from these crystals, upon evaporation at 45° under diminished pressure gave a further yield of the same crystalline material. Altogether 3.12 grams of adenine nucleotide were obtained.

In its chemical composition, crystalline form and solubilities, in its characteristic ability to form supersaturated solutions, in the curious behavior of its water of crystallization and in all other respects the substance is identical with the adenine nucleotide of Jones and Kennedy.



I. 0.3108 required 16.2 cc. of standard  $\text{H}_2\text{SO}_4$ . (1 cc. = 0.003642 N).

II. 0.2374 required 12.32 cc. of standard  $\text{H}_2\text{SO}_4$ .

III. 0.4615 after complete burning gave 0.3105 Mg  $\text{NH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ .

IV. 0.4335 after complete burning gave 0.2892 Mg  $\text{NH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ .

V. 0.3416 heated 2 hours with 5 per cent  $\text{H}_2\text{SO}_4$  gave 0.2292 Mg $\text{NH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$  and 0.3428 adenine picrate.

VI. 0.3001 heated 2 hours with 5 per cent sulfuric acid gave 0.1982 Mg $\text{NH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$  and 0.2994 adenine picrate.

VII. 0.5349 lost 0.0262 at 127°.

VIII. 0.3162 lost 0.0149 at 127°.

|                     | REQUIRED FOR<br>$\text{C}_{10}\text{H}_{14}\text{N}_5\text{PO}_7 \cdot \text{H}_2\text{O}$ | FOUND |       |      |      |       |       |      |      |
|---------------------|--|-------|-------|------|------|-------|-------|------|------|
|                     |  | I     | II    | III  | IV   | V     | VI    | VII  | VIII |
| Nitrogen.....       | 19.18  | 19.00 | 19.02 |      |      |       |       |      |      |
| Total phosphorus... | 8.49   |       |       | 8.51 | 8.44 |       |       |      |      |
| Partial phosphorus. | 8.49   |       |       |      |      | 8.48  | 8.36  |      |      |
| Adenine.....        | 37.00  |       |       |      |      | 37.20 | 36.92 |      |      |
| Water.....          | 4.93   |       |       |      |      |       |       | 4.89 | 4.71 |

The mother liquor from adenine nucleotide of course contained uracil nucleotide. It was allowed to evaporate at the room temperature to about one-fifth of its volume, filtered from a trace, heated and neutralized with brucine in substance. On cooling the deposited crystalline brucine salt was filtered off and recrystallized from 35 per cent alcohol (6.46 grams); 0.6841 required 13.12 cc. of standard acid (1 cc.=0.003642N).

| NITROGEN REQUIRED FOR BRUCINE SALT OF                                   |   | FOUND |
|---|---|-------|
| Adenine nucleotide<br>$C_{10}H_{14}N_5(C_{23}H_{25}N_2O_4) \cdot 7H_2O$ | Uracil nucleotide<br>$C_9H_{13}N_2PO_9(C_{23}H_{25}N_2O_4) \cdot 7H_2O$ |       |
| 9.76  | 6.79  | 6.99  |

It seems a little curious that crystalline insoluble adenine nucleotide never appears until one has prepared a brucine salt of this material. The identical procedure described in this paper for preparing the substance from a mixture of brucine salts had been already executed in preparing the original nucleotide mixture, yet this nucleotide mixture could be evaporated to a syrup without depositing any adenine nucleotide. It might be supposed that an impurity in the nucleotide mixture which prevents the crystallization of adenine nucleotide, is gotten rid of in the mother liquor from the brucine salts. But we were able to prepare crystalline adenine nucleotide from this mother liquor just as from the crystallized brucine salts. Perhaps it is necessary to start with a mixture of the nucleotides, in which adenine nucleotide is in considerable excess.

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# CHANGES IN THE HYDROGEN ION CONCENTRATION OF THE URINE, AS RESULT OF WORK AND HEAT

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## INTRODUCTION

In a previous article (1) I reported on changes in the hydrogen ion concentration of the sweat caused by muscular exercise and heat. At the same time that I was making these determinations I was collecting samples of urine from the subjects in order to ascertain what changes, if any, might be effected in its reaction under precisely like conditions.

Study of the sweat meant working in a quite unexplored field, and this, in part at least, might be said of the experiments that I have to report on the urine, for so far as I have been able to ascertain the effects of heat on the hydrogen ion concentration of the urine have not heretofore appeared in the literature. As to the effects of muscular exercise upon changes in the acidity of the urine, several observers have published their results from time to time. But the fact that the methods that were employed in obtaining the total acidity of urine and similarly constituted fluids are now known to be obsolete, and furthermore that the results reported were somewhat conflicting, suggested a reëxamination of the subject.

The principal observers who have investigated this phase of the problem are Hoffman (2), Ringstedt (3), Oddi and Tarulli (4), Vozarik (6) and Aducco (5). All except the last named have maintained that muscular exercise increased the acidity of the urine; Aducco on the other hand has claimed a decrease in the acidity.

It is well to state at this juncture that in my experiments the subjects were not required to take extremely fatiguing exercise. The work for the time was hard and the heat endured was fairly intense. The subjects remained with me about one hour, which included the time of stripping and dressing.

Exactly the same methods were employed as were used with the sweat, and like precautions were here observed. It seems only necessary to repeat that the hydrogen ion concentrations were obtained by Henderson and Palmer's (7) colorimetric method controlled by occasional electro-metric measurements (Clarke's (8) technique).

#### EXPERIMENTS

As in the case of sweat, my first observations were upon subjects from the Baltimore Central Y. M. C. A. The exercise taken was basket ball, volley ball and the usual work of a gymnasium, which was generally followed by running, these exercises lasting from one-half to one and one-half hours.

It soon became apparent that volunteer subjects in a large city gymnasium were not altogether dependable; consequently it was found much more desirable to have paid subjects come to the laboratory where their part of the work would come under closer scrutiny.

As these experiments were performed at least two hours, and usually five hours, after eating, and as the intervals between taking of the samples were so short, it did not seem necessary to lay much stress on the diet, particularly as it was the relative rather than the absolute values which were most desired.

I took note on all morning experiments when I discovered that the urine had not been voided since breakfast, especially when alkali-producing fruits had been eaten. The main purpose I had in view was to ascertain whether the effects of such diets might be observed in the absolute values. Some of the high alkaline findings may be due to the retention of the morning urine after eating the above type of fruits. On the whole, in my eighty-six observations of normal urines I found the fluctuating values which have been emphasized by many others.

When the experiments were taken up in the laboratory, a stationary bicycle was employed for the muscular exercise and the sweat-cabinet, for the heat. In this connection, however, I might say that heat was not introduced into the experiments with the thought of studying its effect on the urine, but only for the purpose of obtaining heat sweat. It was accidentally and subsequently that I conceived the idea of studying the reaction of the urine under the influence of heat. Consequently, in my first experiments I took only a control sample of the urine and another after the heat sweat had been produced and the muscular exercise had been completed. But after performing three



experiments of this character I decided to test the urine as well as the sweat secreted after heat. So thereafter four samples of urine were obtained at about fifteen-minute intervals. The first sample was taken just before the subject entered the sweat-cabinet, the second just as he came out and was ready to exercise on the bicycle, the third imme-

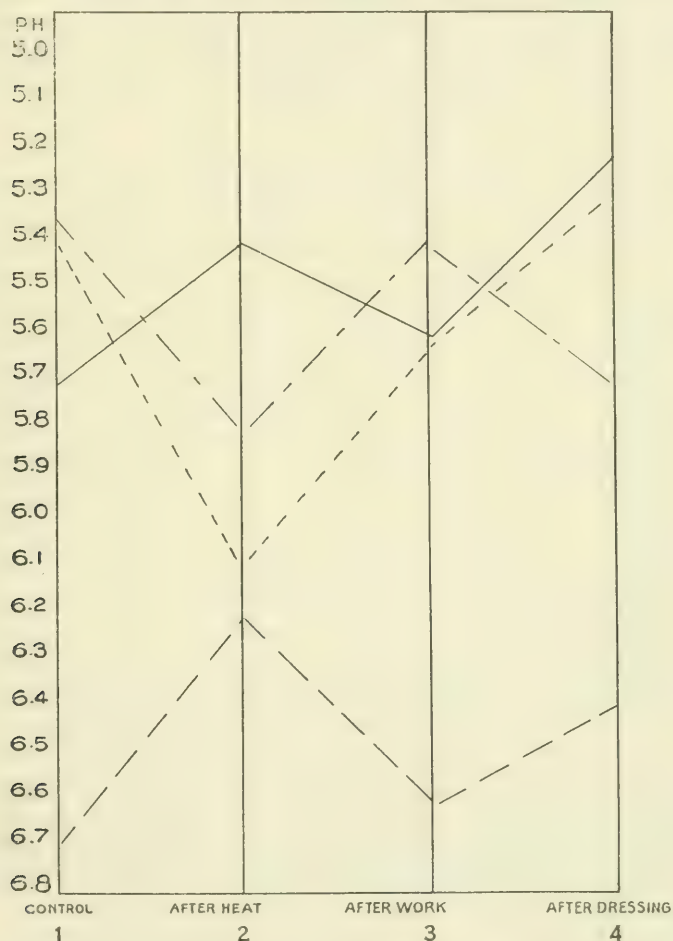


Fig. 1. A chart representing four typical examples of the changes in the hydrogen ion concentrations in urine where work followed immediately after the subjection to heat. Ordinate represents the hydrogen ions in tenths of pH values. Abscissa, the four periods in which urine was taken in 15-minute intervals. Under no. 1, control; under no. 2, after heat; under no. 3, after work; under no. 4, after dressing.

diately after exercise, and the fourth after he had dressed. In this connection it might be stated that the person was subjected to a heat of about  $30^{\circ}\text{C}$ . which rose rapidly to  $40^{\circ}$  or  $45^{\circ}\text{C}$ .

There were in all eleven experiments in which four samples were taken in the above order. The most remarkable facts shown by these tests were that: *a*, there were different concentrations of the hydrogen ions for each of the four periods; *b*, it seemed impossible to establish anything like a fixed relationship between the periods and the H-ion

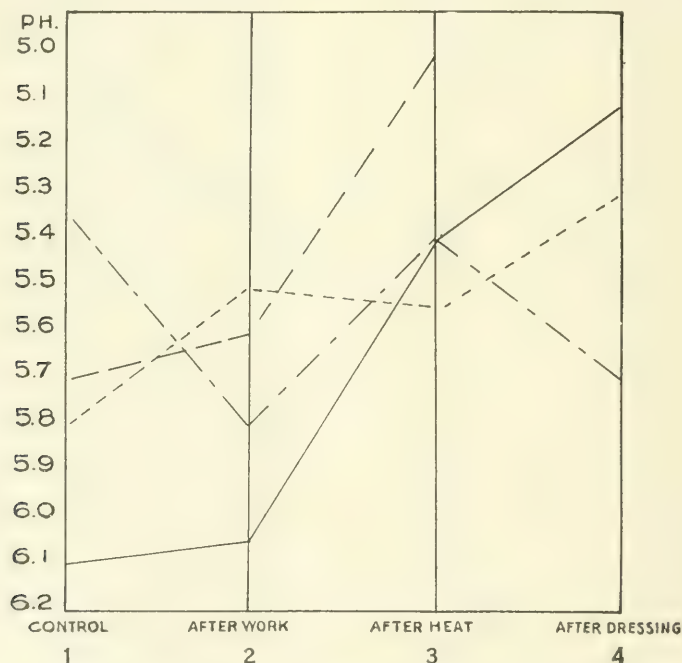


Fig. 2. A chart representing four typical examples of the changes in the hydrogen ion concentrations in urine where heat followed immediately after muscular exercise. Ordinate represents the hydrogen ions in tenth of pH values. Abseissa, the four periods in which urine was taken in 15-minute intervals. Under no. 1, control; under no. 2, after work; under no. 3, after heat; under no. 4, after dressing.

concentration. In most cases the samples obtained in the second, third or fourth period were more acid than was the first sample, which was the control. On the other hand it was most difficult to predict whether the second, third or fourth sample would give the greatest

hydrogen ion concentration. For the sake of clearness I have plotted four typical examples in figure 1, the ordinates representing pH values and the abscissa representing the four samples of urine collected at about fifteen minute intervals.

In the next series of experiments my subjects took the exercise first and then were subjected to heat, which meant that the samples taken at the second and third periods were now reversed. Here again I found just as variable results. In this order ten experiments were performed in which I have plotted four typical cases as shown in figure 2.

These two sets of experiments brought results, as far as urine was concerned, that were difficult to analyze, but yielded some of my most important data on sweat. They seemed to obscure more or less the effects of either work or heat alone upon the urine reaction.

Thereafter the effects of muscular exercise and heat were tested separately. The former will be discussed first. Three samples of urine were now taken: First, just before the exercise, as a control; second, immediately afterwards, and third, after dressing. The results, given in table 1, are listed under subjects G., M. and X. The first two named represent single individuals, while X. represents the report on several individuals. The interval of time between the taking of samples was about twenty minutes.

It is also to be noted that in fourteen experiments with subject G. a greater hydrogen ion concentration was found in the second sample than in the first or control. Two of them show a slight loss while one is unaffected. In comparing the third sample with the control I discovered that twelve out of the fourteen samples had a greater acidity, one sample exhibiting a loss, the other remaining the same. It should also be pointed out that there is only one instance in which the second sample shows the smallest amount of acidity.

In examining the seven experiments performed with subject M., it will be observed that in every instance the urine tested immediately after exercise showed a greater acidity than the control. In the third period four samples are more acid than in the second period, while three show a loss. One of these three is less acid than the control.

In the averages of all the pH values from G., a fairly conspicuous increase in acidity is evident immediately after the exercise, persisting even to the third period, but not so perceptible as the total pH values of 6.04, 5.76 and 5.61, respectively, indicate.

In examining the total averages in the case of M., one observes a difference of 0.3 pH between the first and second periods, with a difference only of 0.03 between the second and third.

Under X. are sixteen experiments in which there were taken only a control and a sample immediately after, corresponding to the second

TABLE 1

| SUBJECT   | URINE<br>CONTROL | URINE<br>WORK | URINE<br>20 MINUTES<br>AFTER | SUBJECT  | URINE<br>CONTROL | URINE<br>WORK | URINE<br>20 MINUTES<br>AFTER |
|-----------|------------------|---------------|------------------------------|----------|------------------|---------------|------------------------------|
|           | pH               | pH            | pH                           |          | pH               | pH            | pH                           |
| G.        | 5.65             | 5.6           | 5.5                          | M.       | 7.0              | 6.7           | 5.8                          |
| G.        | 5.5              | 5.55          | 5.55                         | M.       | 7.4              | 7.35          | 7.6                          |
| G.        | 5.9              | 5.4           | 5.3                          | M.       | 7.5              | 7.0           | 7.5                          |
| G.        | 5.9              | 5.1           | 5.8                          | M.       | 6.9              | 6.85          | 6.8                          |
| G.        | 7.0              | 6.2           | 5.8                          | M.       | 8.5              | 7.9           | 7.7                          |
| G.        | 5.6              | 5.5           | 5.45                         | M.       | 6.8              | 6.4           | 6.3                          |
| G.        | 5.9              | 5.7           | 5.7                          | M.       | 6.7              | 6.1           | 6.4                          |
| G.        | 6.0              | 6.1           | 6.0                          |          |                  |               |                              |
| G.        | 8.7              | 7.7           | 7.0                          | Av. pH.. | 7.2              | 6.9           | 6.87                         |
| G.        | 5.8              | 5.7           | 5.6                          |          |                  |               |                              |
| G.        | 5.75             | 5.75          | 5.35                         | X.       | 6.0              | 5.3           |                              |
| G.        | 5.8              | 5.5           | 5.3                          | X.       | 5.2              | 5.1           |                              |
| G.        | 5.7              | 5.6           | 5.0                          | X.       | 5.6              | 5.1           |                              |
| G.        | 5.4              | 5.3           | 5.2                          | X.       | 7.4              | 6.0           |                              |
|           |                  |               |                              | X.       | 7.4              | 6.9           |                              |
| Av. pH... | 6.04             | 5.76          | 5.61                         | X.       | 6.2              | 5.9           |                              |
|           |                  |               |                              | X.       | 5.7              | 5.3           |                              |
|           |                  |               |                              | X.       | 8.0              | 4.7           |                              |
|           |                  |               |                              | X.       | 7.2              | 5.3           |                              |
|           |                  |               |                              | X.       | 7.2              | 6.5           |                              |
|           |                  |               |                              | X.       | 5.9              | 5.7           |                              |
|           |                  |               |                              | X.       | 6.1              | 6.15          |                              |
|           |                  |               |                              | X.       | 6.0              | 5.7           |                              |
|           |                  |               |                              | X.       | 8.0              | 5.6           |                              |
|           |                  |               |                              | X.       | 5.55             | 5.5           |                              |
|           |                  |               |                              | X.       | 6.2              | 6.0           |                              |
|           |                  |               |                              | Av. pH.. | 6.48             | 5.67          |                              |

period in the other experiments. Of these sixteen determinations, fifteen showed a decided increase in acidity as a result of work, while one shows a decrease of 0.05 pH. The averages here show 6.48 pH for control against 5.67 pH immediately after the exercise. These figures are very significant, and they appear all the more so when one notes the summary of thirty-seven observations as shown in table 2.



In the same manner the effects of heat were studied in order to show how heat may modify the changes of the hydrogen ion of the urine. In table 3 G., M. and X. signify the same subjects as before. In the case of subject G., in fifteen out of eighteen tests the second sample yielded an increase of acidity over the control, in test 2 it showed a slight loss, and in one it remained unaffected. In five instances the second sample was more acid than the third, while in eight cases the third sample was of greater acidity than the second; and in five the acidity was unchanged.

An examination of the results from subject M. show relatively about the same changes. As regards the miscellaneous subjects under X., comparable results were not obtained on account of the fact that the tendency to an increased acidity is delayed to the last period.

TABLE 2  
*Averages of pH values as affected by work*

| OBSERVATIONS | SUBJECTS      | CONTROL | WORK | AFTER |
|--------------|---------------|---------|------|-------|
|              |               | pH      | pH   | pH    |
| 14           | G.            | 6.04    | 5.76 | 5.61  |
| 7            | M.            | 7.2     | 6.9  | 6.87  |
| 16           | X.            | 6.48    | 5.67 |       |
| 37           | Averages..... | 6.57    | 6.11 | 6.24  |

An examination, however, of all the individual tests coupled with the general averages as shown respectively in table 3 and table 4 forces one to the conclusion that heat has a tendency to increase the elimination of acids through the urine. Out of thirty-one observations my results show twenty-nine with an increased acid elimination manifested either in the second or the third sample. The two exceptions are found under X.

These results, then, are quite as striking as those obtained from the effects of exercise and, like the latter, are more significant when one considers the short duration of the experiments and the fact that the subjects were not exposed to excessive heat.

The exact changes that muscular exercise may bring about in the urine by causing an increase of total acidity constitute a question that only further research can settle. It was once thought that, at least as far as normal urines were concerned, the variations of acidity might be explained by the relative proportions of the mono- and di-acid phosphates.

TABLE 3

| SUBJECT   | URINE<br>CONTROL | URINE<br>HEAT | URINE<br>20 MINUTES<br>LATER | SUBJECT  | URINE<br>CONTROL | URINE<br>HEAT | URINE<br>20 MINUTES<br>LATER |
|-----------|------------------|---------------|------------------------------|----------|------------------|---------------|------------------------------|
|           | pH               | pH            | pH                           |          | pH               | pH            | pH                           |
| G.        | 5.8              | 5.9           | 5.6                          | M.       | 6.4              | 5.8           | 6.0                          |
| G.        | 5.6              | 5.45          | 5.5                          | M.       | 5.15             | 5.05          | 5.05                         |
| G.        | 5.6              | 5.5           | 5.6                          | M.       | 5.95             | 5.9           | 5.8                          |
| G.        | 5.75             | 5.5           | 5.85                         | M.       | 6.05             | 5.25          | 5.25                         |
| G.        | 5.75             | 5.7           | 5.8                          | M.       | 7.4              | 7.4           | 7.1                          |
| G.        | 6.8              | 6.0           | 6.0                          | M.       | 8.0              | 7.6           | 7.4                          |
| G.        | 5.9              | 5.8           | 5.85                         | M.       | 6.7              | 6.1           | 6.4                          |
| G.        | 6.2              | 6.0           | 5.6                          |          |                  |               |                              |
| G.        | 7.6              | 7.4           | 7.3                          | Av. pH.. | 6.52             | 6.16          | 6.14                         |
| G.        | 6.0              | 5.9           | 5.9                          |          |                  |               |                              |
| G.        | 5.5              | 5.55          | 5.45                         |          |                  |               |                              |
| G.        | 5.6              | 5.5           | 5.4                          | X.       | 5.7              | 5.2           | 5.1                          |
| G.        | 5.7              | 5.6           | 5.65                         | X.       | 6.2              | 6.6           | 6.1                          |
| G.        | 5.4              | 5.2           | 5.2                          | X.       | 7.5              | 7.0           | 5.2                          |
| G.        | 5.8              | 5.6           | 5.6                          | X.       | 6.2              | 5.7           | 5.6                          |
| G.        | 5.75             | 5.75          | 5.35                         | X.       | 7.1              | 8.7           | 8.2                          |
| G.        | 5.8              | 5.4           | 5.3                          | X.       | 5.8              | 6.0           | 6.0                          |
| G.        | 5.7              | 5.0           | 5.0                          |          |                  |               |                              |
| Av. pH... | 5.9              | 5.71          | 5.66                         | Av. pH.. | 6.42             | 6.5           | 6.03                         |

TABLE 4

*Averages of pH values as affected by heat*

| OBSERVATIONS | SUBJECTS      | CONTROL | HEAT | AFTER |
|--------------|---------------|---------|------|-------|
|              |               | pH      | pH   | pH    |
| 18           | G.            | 5.9     | 5.71 | 5.66  |
| 7            | M.            | 6.52    | 6.16 | 6.14  |
| 6            | X.            | 6.42    | 6.5  | 6.03  |
| 31           | Averages..... | 6.28    | 6.12 | 5.94  |

Some years ago Folin (9) and also Henderson (10) called attention to the inadequacy of such an explanation. Folin states:

The current attractive, and in a measure plausible, belief that the acidity of urine is regulated by variations in the relative proportion of the two forms of 'acid phosphates' is therefore erroneous. If urine does at no time contain comparatively strong acids in the free form, the reason is in part the variability of the ammonia formation and in part the presence of salts of organic acid.

### Henderson states:

If phosphoric acid be the chief substance which is varying in amount of base associated with it as the reaction of the urines varies, it is none the less true that other substances are varying too. However, only two, so far as we now know, will at the ordinary physiological ranges show considerable change in combined base. These substances are carbonic acid and uric acid, both of which occur in such small amount as to be of minor importance.

In the examination of Scaffidi's (11) work, which bears directly on the problem, one is led to believe that while the uric acid is normally quite negligible it may become something of a factor as a result of muscular fatigue. While he says that during the exercise there is no increase in uric acid, at the same time he states:

Immédiatement après l'interuption du travail musculaire, il se manifeste une augmentation plus ou moins considérable, mais en tout cas très rapidé et très sensible, dans l'élimination de l'acide urique. Les bases puriniques, dans cette période diminuent parfois. L'augmentation dans l'élimination de l'acide urique est beaucoup plus sensible quand la fatigue musculaire, et spécialement le repos consécutif, ont lieu à des altitudes qui ne sont pas très grandes.

Kenneway (12) has obtained the same results, with this difference: the increase following exercise is only of importance when unaccustomed muscles are brought into use. He claims that after frequent use of these muscles the elimination of uric acid becomes less in amount. Furthermore, he holds that the increased excretion of uric acid immediately following work is not due to the "sweeping out of that which is already present and would otherwise be retained, but that exercise heightened the activity processes by which uric acid is produced," and maintains that the decrease of elimination during work is due to defective oxidation of purine compounds.

Hill and Flack (13) have stated that during severe muscular exercise "the use of oxygen and production of  $\text{CO}_2$  are so rapid in the muscles that the circulation cannot keep pace with the demand." Ryffel (14) claimed that the presence of lactic acid in the urine during exercise might be regarded as indicating the inadequacy of the supply of oxygen.

My data are not in harmony with Kenneway's that the continued use of the same set of muscles decreases the elimination of uric acid and that the increase is found only as a result of the use of an unaccustomed set. In my experiments the exercise was done almost entirely on the stationary bicycle, and no depreciation in the acidity resulting from constant use of the same set of muscles.

As to the causes of increased acidity of the urine as a result of heat, we are even more in the dark.

Finally, it should be mentioned that my results conflict with the statement of Vozarik (15) that changes in external temperature, as warm baths, decrease the acidity of the urine.

#### SUMMARY

1. Intense exercise from fifteen to twenty minutes' duration increases the hydrogen ion concentration of the urine.

2. A man subjected to the heat of a sweat-cabinet for fifteen or twenty minutes will, in a large majority of cases, void a urine of higher hydrogen ion concentration.

3. If muscular exercise follows immediately upon subjection to heat, or vice versa, it is impossible to interpret the results.

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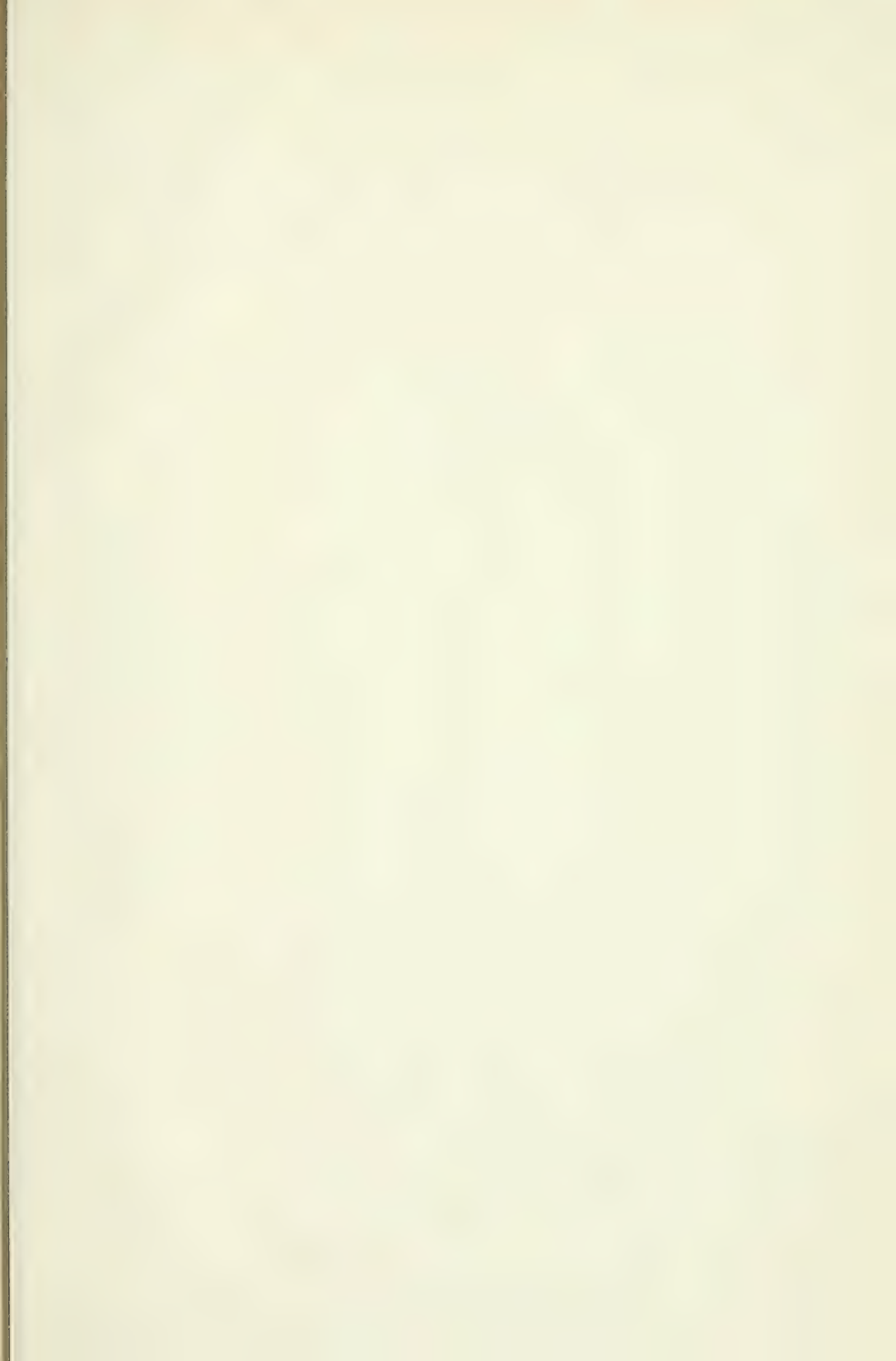
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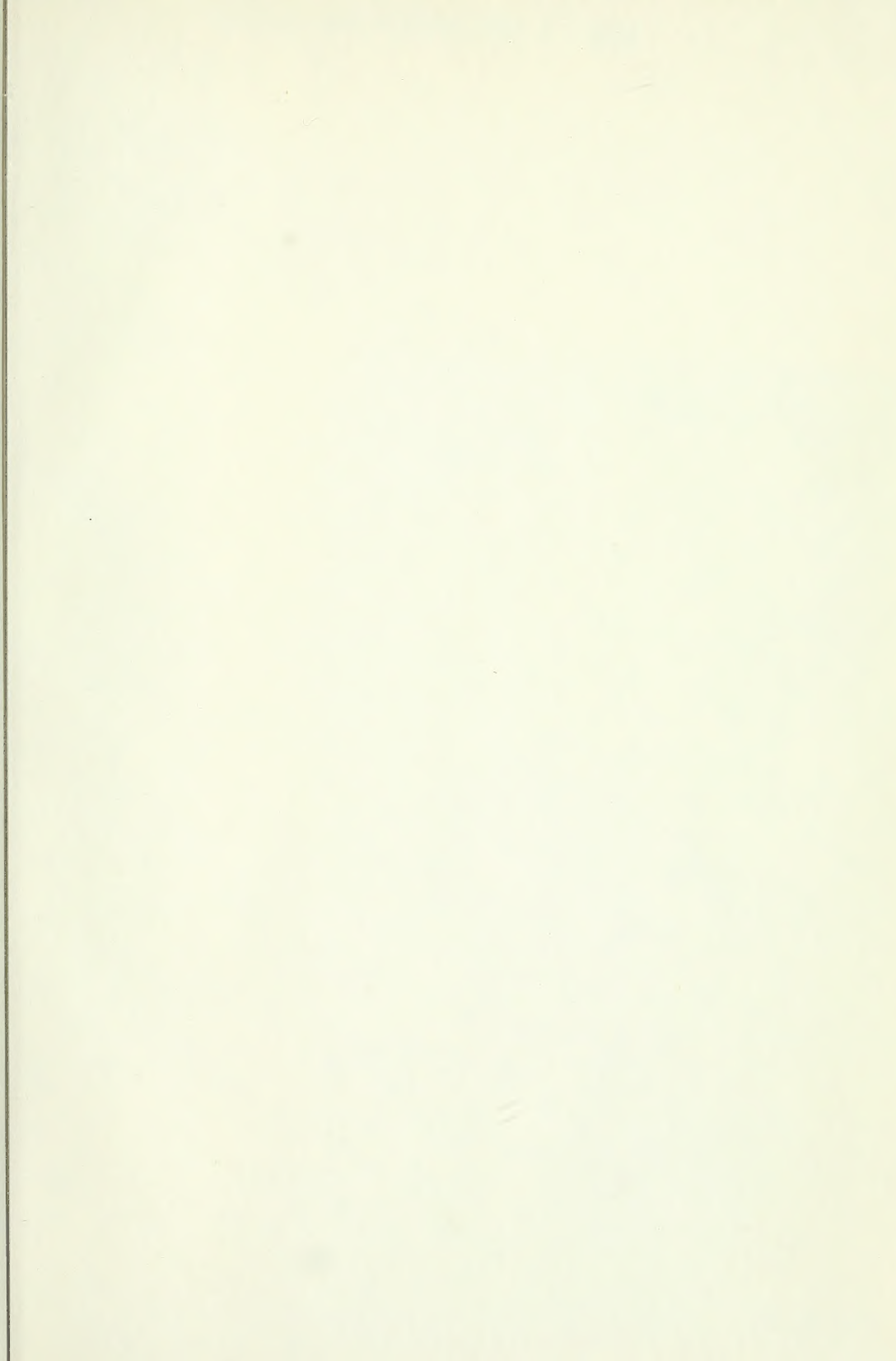
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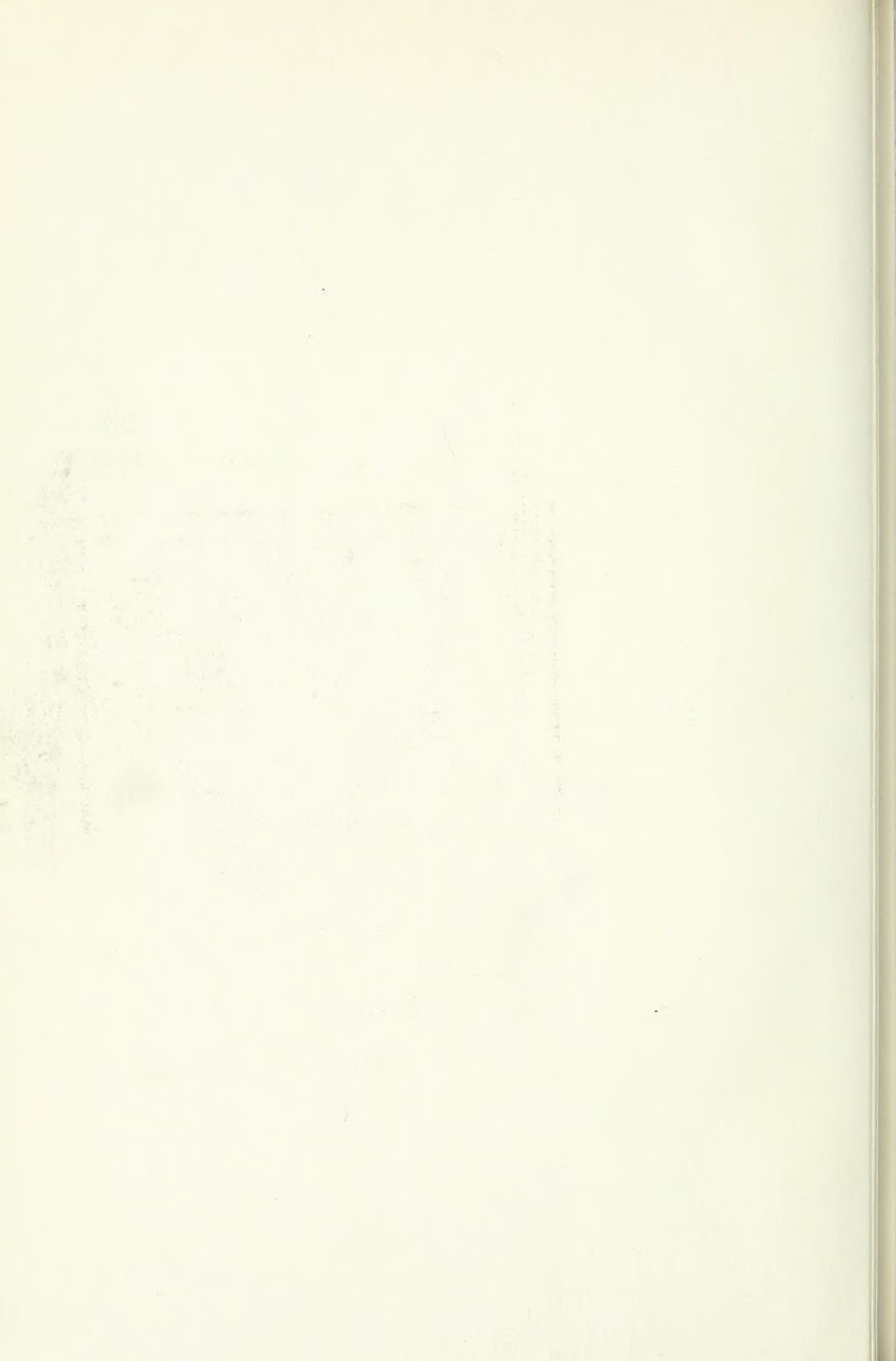
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